UNIVERSITY OF BOTSWANA

EVALUATION OF NUTRITIONAL VALUE OF SPENT OYSTER MUSHROOM (*PLEUROTUS* SPP.) SUBSTRATE - BASED DIETS AND NUTRIENT DIGESTIBILITY BY TSWANA LAMBS

MASTER OF SCIENCE IN ANIMAL SCIENCE

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Evaluation of nutritional value of spent oyster mushroom (*Pleurotus* spp.) substrate-based diets and nutrient digestibility by Tswana lambs

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DECLARATION

The work contained in this dissertation was compiled by the author at Botswana University of Agriculture and Natural Resources, during the period of January 2016 to June 2019. It is my original work and all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. It has not been submitted and shall not be submitted for the award of any other degree or diploma of any other university.

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"I will be glad and rejoice in You; I will sing praise to Your name, O most high" (Psalm 9:2).

DEDICATIONS

I dedicate this work to my mother, who I express my great sense of honour, love and earnest gratitude for her needy inspiration throughout the tough and straining period of this research work.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degrees Celsius
3, 4- DHP	3,4-dihydroxypyridine
ADF	Acid Detergent Fibre
ADG	Average Daily Gain
ADL	Acid Detergent Lignin
AFRC	Agricultural and Food Research Council
APRU	Animal Production Research Unit
BAMB	Botswana Agricultural Marketing Board
BUAN	Botswana University of Agriculture and Natural Resources
Ca	Calcium
CH_4	Methane
CIOMS	Council for International Organisation of Medical Sciences
CP	Crude Protein
CRD	Completely Randomised Design
CV	Coefficient of Variation
DAC	District Agricultural Coordinator
DCP	Di-Calcium Phosphate
DFI	Daily Feed Intake
DM	Dry Matter
EE	Ether Extract
EMU	Estate Management Unit
FAO	Food and Agriculture Organization

FCR	Feed Conversion Ratio
G/kg	Grams per kilogram
GDP	Gross Domestic Product
H_2SO_4	Sulphuric acid
Ha	Hectare
LSD	Least Significance Difference
Mg/kg	Milligrams per kilogram
ML	Millet stover based-diet
MLSS	Millet Spent Substrate based-diet
MS	Maize Stover based-diet
MSSS	Maize Spent Substrate based-diet
NDF	Neutral Detergent Fibre
NRC	National Research Council
P	Phosphorus
RDP	Rumen Degradable Protein
S	Sorghum stover based-diet
SAS	Statistical Analysis Software
SEM	Standard Error of the Mean
SMS	Spent Mushroom Substrate
SOMS	Spent Oyster Mushroom Substrates
SPSS	Statistical Package for Social Sciences
SSS	Sorghum Spent Substrate based-diet
TMR	Total Mixed Ration
VFA	Volatile Fatty Acids

DECLARATION

The research described in this thesis was carried out in Department of Animal Science and Production, Botswana University of Agriculture and Natural Resources, Gaborone under the supervision of Professor O.R. Madibela, Professor E.B. Khonga and Dr T.V. Balole.

This is to declare that this thesis is the result of my own investigation and had not been presented in any previous application for a degree. All sources of information are shown in the text and listed in the references and all assistance by others has been duly acknowledged.

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Signed………………………………. Date…………………….

I, Professor Madibela, approved the release of this thesis for examination

Signed…………………………. Date…………………….

I, Professor Khonga, approved the release of this thesis for examination

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I, Dr Balole, approved the release of this thesis for examination

Signed…………………………. Date…………………….

GENERAL ABSTRACT

Cereal stover contributes immensely to ruminant nutrition but are harvested when they are low in nutritive value. This affects rumen fermentation and animal productivity. Bio-conversion of cereal stover through mushroom may increase its nutritive value index. This dissertation was a record of three studies. The first study was a survey that assessed the current uses and yields of crop residues by small-scale farmers in Kgatleng, Kweneng and Southern districts of Botswana. Survey data were collected through structured questionnaires that were administered face to face to the identified small-scale farmers in the mentioned districts. Survey questionnaire data were analysed using Statistical Package for Social Sciences software. Farming and utilisation of crop residues were dominated by male farmers 58.7% (37/63). Most of the farmers 66.7% (42/63) harvested and stored cereal crop residues for feeding animals after harvesting grains while 33.3% (21/63) left the crop residues to be grazed *in situ* by livestock. Cattle and goats were the most (50.8%) animals that benefited from feeding with crop residues. Maize crop residues were perceived to be of very poor quality, sorghum crop residues rated better in terms of nutritive value while 98.4% (62/63) of the farmers did not know the quality of millet crop residues. In trying to improve the value of crop residues, farmers added feed ingredients such as molasses, salt, and wheat bran while cowpeas stover and lablab were added to improve protein content of the crop residues. The second study was conducted to determine the effect of crop residue type on the nutritional composition of oyster mushroom spent substrate-based diets for growing Tswana lambs. The study was arranged in a 3 x 2 factorial design with residue type (millet stover, sorghum stover and maize stover) as main plot (Factor A) and crop residue treatment (mushroom spent substrate and untreated) as subplot (Factor B). Six treatment diets were formulated by mixing 55.0% of each of millet spent substrate based-diet (MLSS), sorghum spent substrate based-diet (SSS), maize spent substrate based-diet (MSSS), millet stover based-diet (ML), sorghum stover based-diet (S) and maize

stover based-diet (MS) to 45% of other ingredients to achieve a total mixed ration. The nutritive values of the diets were determined using AOAC procedures while *in vitro* dry matter digestibility (IVDMD) of the diets was determined through incubation of test diets in Daisy^{II} incubator for 48 hours and *in sacco* degradability of dry matter (DM) was determined by incubating test diets in the rumen of two cannulated steers for 3, 6, 12, 24, 48, 72 and 96 hours. Data were analysed using General Linear Model procedures in Statistical Analysis System (SAS). There were no significant differences (P>0.05) in crude protein (CP) contents (15.56 and 15.42%) between mushroom treated and untreated substrate based-diets respectively, while mushroom treated substrate based diets had significantly lower (P<0.05) acid detergent fibre (ADF) (15.88% compared to 18.11% of the untreated diet) and acid detergent lignin (ADL) contents (7.16% compared to 8.65% of the untreated diet). There was an increase in IVDMD (from 79.72 to 85.57%) and organic matter digestibility (from 87.06 to 89.06%) due to the mushroom treatment effect. Significant interaction effect (P<0.05) was observed in CP with SSS having the highest content (15.92%) and lowest in other diets (15.09-15.67%). Fat (13.96%), percentage digestible energy (92.79%) and gross energy (21.96 MJ/Kg) were high (P<0.05) in SSS, MSSS and ML respectively while OMD was high in MSSS (90.06%) and lowest in ML (84.68%). Treatment of crop residues increased (P<0.05) quickly digestible fraction (*a*), potential degradability (PD) and effective degradability ($ED_{0.03}$ and $ED_{0.05}$) of dry matter, which is likely to increase the supply of nutrients to the animal and improve productivity. The highest degradability response was for MSSS while ML had the lowest degradability parameters. The third study determined the effects of mushroom spent substrates based diets from local cereal crop residues on *in vivo* nutrient digestibility, nitrogen balance and growth of Tswana lambs. Twelve castrated Tswana lambs aged between 9-12-months were randomly assigned to three dietary treatments in a completely randomised design with four lambs per treatment: MS, MSSS and SSS for 42 days. Data were analysed using General Linear

Model procedures in SAS. Treatment effect showed no significant difference ($P>0.05$) on final body weight (FBW), feed conversion ratio (FCR), total weight gain (TWG) and average daily gain (ADG). Lambs fed SSS and MSSS diets had significantly higher daily feed intake (DFI) ($P<0.05$) of 586.00 g/day and 581.50 g/day respectively than lambs fed MS diet (464.70 g/day). Significant differences ($P<0.05$) were observed in nutrient digestibility of Dry matter; MS, MSSS and SSS (55.84%, 51.72% and 48.12% respectively). acid detergent fibre (ADF) digestibility was higher in lambs fed MS (51.82 %) and lowest in lambs that consumed MSSS (44.50%) while acid detergent lignin (ADL) digestibility was highest ($P<0.05$) in lambs offered MSSS (29.46%) and SSS (26.36%) diet and lowest in those fed MS (19.33%). Fat (87.89%) digestibility and calcium (Ca) (84.00%) disappearance was significantly higher ($P<0.0001$) in lambs fed SSS diet compared to other diets. Phosphorus disappearance was 95.00%, 91.57% and 88.38% in lambs fed MS, MSSS and SSS respectively. Nitrogen intake of 14.38 and 14.39 g/day were observed in lambs fed MSSS and SSS respectively which were significantly higher ($P<0.05$) than of lambs fed MS diet. Nitrogen balance (12.23 and12.50 g/day) and retention (8.80 and 9.06 g/day) were also high in lambs fed MSSS and SSS respectively. The inclusion of mushroom spent substrates in complete diets did not support better growth performance of lambs but improved feed intake and digestibility of some nutrients such as ADF, ADL, fat and Ca disappearance by lambs. High inclusion rate (55%) could have limited high growth rate of Tswana lambs, therefore future research may test low inclusion rate of spent mushroom substrates together with small amounts of high quality protected protein such as fish meal or fatty acid coated protein that escapes rumen degradation in complete diets, to promote desired growth.

Keywords: growth performance, mushroom spent substrates, nitrogen balance, nutrient digestibility, Tswana lambs

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Livestock feeding in Botswana

According to Burgess (2006), natural pasture is the predominant and cheapest feed source for livestock in most areas of Botswana. With Botswana being characterised as a semi-arid country, receiving highly variable rainfall (Adaptation at scale in Semi-arid regions, 2015), this causes availability and quality of pasture and production of green fodder to be low (Madibela *et al*., 2002) and not able to meet nutritional needs of livestock resulting in poor livestock production. Madibela *et al*. (2002) stated that in Botswana, pasture is not always available throughout the year but only for a period of five months. With a limited amount of quality pasture all year-round, the health status and productivity of animals is compromised (Khan *et al*., 2009).

To reduce reliance on natural pasture as a source of feed for animals, farmers can produce fodder for their livestock. However, with an extra land requirement, water shortage, more labour requirement (Naik *et al*., 2015) for fodder production, farmers who venture into livestock production do not pay adequate attention to the production of fodder. In contrast, farmers producing arable crops do not consider fodder production as a component of their farming venture. Linking the two farming systems; crop and livestock systems are likely to improve overall productivity. Fodder as a by-product from arable farming for livestock is normally harvested at full maturity after seed harvest resulting in poor quality (Dung *et al*., 2010). Khan *et al*. (2009) stated that poor nutrition from such poor forage results in low production such as slow growth rate, loss of body condition, reduced reproductive performance and increased susceptibility to diseases and parasites. With low pasture quality and quantity being the main limiting factor in improving the livestock productivity in Botswana, some farmers extend grazing seasons through feeding crop residues which are collected after harvesting of grains but the systems are not widely practised (Brithal and Jha, 2005). These crop residues are valuable feed resources which in most cases are underutilised.

1.2 Opportunities for improvement of fodder resources

1.2.1 Crop residues

Ruminant animals in most parts of Botswana subsist mainly on crop residue-based diets during the dry period. In most cases, this is achieved by letting animals into crop fields after grain harvest to graze the crop residues, which is wasteful as animal trample on most of the forage (Madibela and Lekgari, 2005). The increasing expansion of agricultural activity over the last few years has led to the accumulation of a large quantity of lignocellulosic residues in the country. Most farmers utilise cereal straws to feed their livestock (Madibela *et al*., 2002) with sorghum, millet and maize stover as basal diet, while cowpea and groundnut hulls are fed as a protein supplement (Dipheko, 2015). Raihanatu *et al*. (2011) stated that other agricultural by-products such as brans and oil cakes are used as sources of energy and mineral supplement after crops are processed. However, some of these products (example: maize stover) are characterised by low nutritive value because of their low digestibility (Shreck *et al*., 2011). Although a vast nutrient potential is locked in these lignocellulosic crop residues (Fazaeli, 2007), they are not utilized to their fullest potential for ruminant feeding due to poor protein, low nitrogen and mineral contents (Ramana Reddy *et al*., 2015).

Improving the nutritive value of crop residues

In trying to improve the nutritive value of crop residues, efforts such as the use of chemicals like ammonia (Kim and Lee, 2005), urea NaOH and CaOH (Meng, 2002) have not been common because they are expensive, not environmentally friendly and not easily accessible.

Recently in Botswana Abdulazeez *et al*. (2016) attempted to treat maize cobs with a wood-ash solution in combination with urea and find variable effects on nutritive value of maize cobs for the different proportions of urea and wood-ash tested. These treatments are meant to get hemicellulose hydrolysed to form fermentable sugars resulting in an increase in rate and digestion of cellulose and hemicellulose (Chaturvedi and Verma, 2013). Physical processing like grinding (Taylor and Bryant, 2007) is employed. Recently, biological treatments of crop residues to improve the accessibility of cellulosic biomass, thus improving their digestibility and feeding value have been tried in developed countries but not in Botswana hence generating extensive interests among researchers. The potential of biological treatments has been described as the ability of certain microbes (specifically fungi) to digest plant cell wall by partial breakdown of the lignin-carbohydrate complex (Keller *et al*., 2003) thus improving their utilization in the rumen by increasing the availability of fermentable energy to ruminal microbes (Akin *et al*., 1993).

The microbial conversion of crop residues into value-added feed products (Villas-Bôas *et al*., 2002) can be achieved by incorporating crop residues into the production of oyster mushrooms by small-scale farmers. This can drastically improve farmers' income through mushroom sales as well as selling or utilizing nutritionally improved crop residues. Crop production in Botswana has shown a decline due to its reliance on rainfall which varies in distribution and amounts. For instance, the average annual rainfall ranges from 660 mm in the northeast of the country to 500 mm in the south-west (Nkemelang *et al*., 2018). According to Statistics Botswana (2014), cereal yield from 1979 to 2012 was variable but showing a declining trend. In 1979 sorghum, maize and millet yields were 90, 80 and 30 kg/hectare (that is P180, P160 and P90 per hectare of income if sold to BAMB) respectively. In 2007, about 40, 70 and 50 kg/hectare was recorded for sorghum, maize, and millet respectively, while during the 2008/2009 to 2011/2012 ploughing seasons, average yields were 110.5 kg/ha, 124.6 kg/ha and

93.4 kg/ha, respectively. For the 2012/2013 season, 53 kg/ha, 144 kg/ha and 154 kg/ha (that is P159, P432 and P462 of income if grains were sold to BAMB at P3/ Kg), respectively. From these statistics, farmers' income is very low despite moderate improvement in yield per hectare because the costs of producing these grains are high (P600/ha) hence some farmers improve their income by selling crop residues. Crop residues which are often left in the field or burnt (Madibela and Lekgari, 2005) by small-scale farmers can be used to produce mushrooms by employing oyster mushroom production. Hence farmers can make an additional income after selling mushrooms after a period of three months at P100/kg compared to P3/kg of grains. Also, farmers can sell crop residues of improved nutritive value at a better price. Conversely, farmers can now afford to buy fertilisers to improve their crop yields, pay their children's school fees through more income generated from selling mushrooms. Lastly, they can also feed their livestock improved crop residues (spent substrates), which they can supplement with other feed ingredients as they will have the capital to buy supplements leading to improved animal performance and more income if these animals are sold to the market.

1.2.2 Spent oyster mushroom substrates

An alternative option besides using crop residues directly as animal feed is to use them in mushroom production and the resulting mushroom spent substrate fed to livestock (Akinfemi and Ogunwole, 2012). With this idea, livestock nutrient demand can be partially met even during the dry season in Botswana through increased biomass availability from treated crop residues. Moreover, due to increased feed cost (Naik *et al*., 2015), interest in addition of agricultural by-products to animal diets has increased. As stated by Oh *et al*. (2010) spent oyster mushroom substrate (SOMS), a by-product generated from the cultivation of Oyster mushrooms is an agricultural by-product of interest. SOMS have been reported to be used in ruminants' diets with a decrease in fibre as a result of oyster mushroom treatment (Kim *et al*., 2010; Oh *et al*., 2010; Xu *et al*., 2010). These studies suggest that the use of SOMS in ruminant diets is possible and could be considered in Botswana.

Annually about 300,000 metric tons (Forestnews, 2011) of spent mushroom (*Pleurotus* spp.) substrates from rice straw, sawdust, corn cobs, rice bran, wheat bran, beet pulp, and bean curd (Park *et al*., 2012) are produced in South Korea and are used as alternative feed resource which can be tried and implemented in Botswana. According to Kim *et al*. (2010), SOMS normally present an economic problem due to associated disposal costs. SOMS is commonly made from renewable agricultural residues such as sawdust, corn cobs, millet, sorghum and maize stover, sugarcane bagasse, oil palm, wheat straw, hay, cottonseed meal, cocoa shells, gypsum and other substances (Jordan *et al*., 2008). SOMS from agricultural residues such as corn-cob and rice bran are reported to have a chemical composition of 55.0% acid detergent fibre (ADF), 73.6% neutral detergent fibre (NDF), 2.1 % ether extract (EE), 6.4% crude ash, 9.8% non-fibrous carbohydrate (NFC) and 8.1 % crude protein (CP), (Kim *et al*., 2007), and based on this, the SOMS has potential as a feed resource for livestock.

The improved nutritive value of SOMS was supported by Sanchez (2010) who stated that edible mushrooms can be cultivated on agricultural residues, such as rice straw, rice bran and sugarcane bagasse and the resulting spent substrate was suitable for consumption by livestock. The reason behind the improved nutritive value of SOMS is because of the fungi, which have the capability to colonize organic materials such as crop residues and forestry wastes resulting in improved feed nutritional value (Ayala *et al*., 2011). Due to enzymatic conversion processes during mushroom cultivation, these substrates are easily utilised by ruminants as feed. The mushrooms degrade lignocellulose in crop residues, making them be used as an appropriate forage source in maintenance rations for ruminants, possibly due to reduced levels of fibre (18-25 g/kg DM) and improve protein content (Kim *et al*., 2010). Silvana *et al*. (2006) reported an increase in digestibility of straws after conversion with oyster mushroom (*Pleurotus* spp.).

The high digestibility of spent substrate is because most of the cell wall components are degraded by enzymes secreted during mycelium growth and mushroom production. There is an increase in crude protein, fat content and ash content of the spent substrates with time, with the most remarkable change of composition in the spent straw substrate being the reduction of hemicellulose by 17 %, cellulose by 15 % and lignin by 4 % (Zhu *et al*. 2012). Medina *et al*. (2009) stated that SOMS have elevated amounts of polysaccharides, vitamins and some trace elements such as Fe, Ca, Zn and Mg, which are valuable for animals. With this improved nutritive value of SOMS, they are a potential source of feed for ruminants. Due to enzymatic conversion processes during mushroom cultivation, SOMS could be more easily digested by ruminants (Akinfemi *et al*., 2009). The white-rot fungi degrade lignin and improve the *in vivo* dry matter digestibility of lignocellulosic materials (Mahesh, 2012). Akinfemi *et al*. (2009) also reported improved digestibility of maize straw after incubation for 40 days, converting it into valuable ruminant feedstuff, using an oyster mushroom. Since SOMS cannot provide every nutrient at a required amount for a particular class of animals, they are normally mixed with other feed ingredients to meet animal's nutrient requirement.

1.3 Use of spent oyster mushroom substrates in formulating diets for small stock

From literature (Mahesh and Mohini, 2013) colonization of crop residues by *Pleurotus* spp. not only improves their nutritive value content but also enhance digestibility, thus making SOMS potential feed resources for ruminant animals which could be used in combination with other feedstuffs. A report by Statistics Botswana (2015), shows that out of 52.959, 18.690 and 71.369 cattle, goats and sheep respectively, only 12.894, 17.811 and 65.853 of grazing cattle, goats, and sheep respectively were supplemented in different regions of Botswana. Just like natural pasture which is high in roughage, SOMS should also be supplemented if they are to be used as feed (Kim *et al*., 2011a). Fazaeli and Masoodi (2006) emphasised that the other reason for SOMS to be supplemented is because they have restricted use as animal feed due to their high crude ash content (35g/100g). Kim *et al*. (2011b) stated that due to mycelial action on the mushroom substrates, alteration of solubility and degradability of their nutrients in the rumen may occur. Therefore, SOMS-based diets can result in an improved feed-nutritional value in ruminants (Kim *et al*., 2011b).

Improved nutritive value of SOMS will ensure that ruminant animals nutrient requirements are met hence SOMS can be used to formulate SOMS based diets which will provide all nutrients needed for animal growth, maintenance or production (McDonald *et al*., 2011). Ramana Reddy *et al*. (2015) stated that formulating diets for ruminant animals using treated crop residues is not an easy task as factors like species of animal, nutrition level of residues, digestibility and voluntary intake must be considered carefully. Kim *et al*. (2010) reported that rice SOMS formulated diet had an increased dry matter intake (DMI) of 7 to 15%. The increase in total DMI reflected the high SOMS based-diet intake (Kim *et al*., 2010) because feeding the formulated feed enhanced feed palatability and met their nutrient needs. Nutrient balancing in North America is used to formulate total mixed rations (TMR) for high producing animals. The same approach could be tried and adopted in Botswana using forages like crop residues with different chemical and structural features since concentrates and protein sources are very expensive. There is limited information concerning the digestion dynamics of forages to formulate crop residues based TMR. Therefore, the current study aimed at determining the effect of crop residue type on the nutritive value of spent oyster mushroom substrate- based diets and assess their nutrient digestibility by lambs.

1.4 Justification

Livestock farming and crop production are considered the backbone of the agricultural sector and their contribution to Botswana's economy cannot be neglected. Their contribution to the gross domestic product (GDP) is 3% (Statistics Botswana, 2014), therefore they constitute a foundation of household incomes and food security in Botswana (Bahta *et al*., 2017). The significance of livestock and crop production in Botswana is hampered by challenges like inadequate livestock feeds, livestock diseases, persistent droughts and low erratic rainfall. Persistent drought is a major limitation in animal productivity in Botswana because it has a major impact on livestock pastures and forage crop production and quality, changes in the distribution of livestock diseases and hence increased livestock mortality (Kgosikoma, 2006). The secondary effect of drought is that it changes animal performance such as reduce body condition, reduced reproduction, reduced milk yield which results mainly from alterations in the nutritional environment. Masike and Urich (2008) stated that changes in climate would affect the quality and quantity of forage produced, leading to poor livestock production.

The challenge of inadequate livestock feeds is an immense problem in Botswana. There is a critical shortage of feed supply during the dry season. Moreover, natural pasture does not provide constant quality of nutrients round the year thus aggravating the challenges associated with livestock productivity in Botswana. Therefore, farmers sustain their livestock on cereal straws and other crop residues of poor quality. Research by Mahesh and Mohini (2013); Kholif *et al*. (2014) emphasised that most crop residues and crop by-products have high fibre, low CP and very low minerals and vitamins. Kuhad *et al*. (2013) also emphasised that crop residues have high amounts of cellulose, hemicellulose and lignin. According to Elghandour *et al*. (2014), lignin and cellulose are the most important limiting factors in using crop by-products for animal feeding as they have low digestibility, due to the presence of non-polysaccharide components such as phenolic acids (Kuhad *et al*., 2013). Therefore, crop residues from cereal crops such as sorghum, millet and maize have low palatability while residues of groundnuts and Bambara groundnuts have high palatability and rich in nitrogen content (Nweke and Emeh, 2013) which may represent an important protein supplement when cereal residues are used as feed for ruminants.

In Botswana, most small-scale farmers lack the resources to purchase the technology which can be used to process the residues to improve their value and the crop residues are left in the field to be grazed by livestock or they are burnt or ploughed under (Madibela and Lekgari, 2005). Therefore, this project will be carried out to assess nutritional value-addition to maize, sorghum and millet SOMS after the cultivation of *Pleurotus* spp. With this technology, the product (spent mushroom substrates) will have a potential to be used in rations (Kim *et al*., 2010) to supply needed nutrients for livestock. Reduced feeding costs, lowering feed wastage and improving low-grade fibrous crop residues to supply adequate nutrients can be achieved through the incorporation of crop residues in mushroom production (Mahesh and Mohini, 2013). The research will start with a survey on the uses of cereal crop residues by small-scale farmers and quantification of residues per 100 m^2 of land. This as a means of increasing the overall income from a hectare of cereals whilst enriching the crop residues as feed for small stock. Moreover, the project will provide farmers with information on the value that mushroom can add to the different crop residues hence playing a role in making additional income if mushroom production is adopted as part of the cereal production cycle as the selling price for mushrooms is much higher than that of grains. Alternatively, farmers can sell the crop residues to oyster mushroom farmers thus generating additional income to that obtained from grain sales. Consumption of mushrooms at the household level will provide an additional nutritious component to the diet and health of farmers. By generating income from households, people living in rural and peri-urban areas will be able to raise their living standards.

1.5 Study aim

The project aimed at testing treatment of cereal stover with *Pleurotus* spp on nutritive value, rumen fermentation of complete diets. The effects of experimental diets on body growth rate, nutrients digestibility and nitrogen balance were also investigated.

1.6 Objectives

1. The main objective of the **first study** was to assess current uses and yields of cereal crop residues by small-scale farmers in Kgatleng, Kweneng and Southern Districts of Botswana.

The specific objectives of this study were:

- To assess how small-scale farmers, utilise the crop residues and estimate the amount of crop residues that can be collected from a $100m^2$ area.

2. The main objective of the **second study** was to determine the nutritional composition of oyster mushroom spent substrate-based diets for growing indigenous lambs.

The specific objectives of this study were:

- To determine the effect of crop residue type on dry matter, protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin, Ash, organic matter, organic matter digestibility, fat, hemicellulose, non-structural carbohydrates, gross energy, tannins and minerals of oyster mushroom spent substrate-based diets for growing indigenous lambs.

- To determine the *in vitro* dry matter digestibility and *in situ* rumen degradation of diets based on mushroom spent substrates for growing indigenous lambs.

3. The main objective of the **third study** was to determine the *in vivo* nutrient digestibility, nitrogen balance and growth performance of the oyster mushroom spent substrate based-diets for growing indigenous lambs.

The specific objectives of this study were:

- To determine the *in vivo* nutrient digestibility of lambs fed spent oyster mushroom substrate based-diets.

- To determine daily feed intake and weight gains of indigenous lambs fed spent oyster mushroom substrate based-diets.

1.7 Hypotheses

1.7.1 Hypotheses of the first objective

H_A: Farmers do know the quality of crop residues hence mixing poor crop residues with other feed ingredients to improve their nutritive value.

1.7.2 Hypotheses of the second objective

H_A: There is a difference due to crop residue type on the nutritional composition, mineral contents, *in vitro* dry matter digestibility and *in situ* degradability of oyster mushroom spent substrate-based diets for growing indigenous lambs.

1.7.3 Hypotheses of the third objective

H_A: There is a difference due to crop residue type on *in vivo* nutrient digestibility, nitrogen balance and growth performance of lambs fed spent oyster mushroom substrates based-diets.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Livestock feeding and alternative feed resources available for ruminant livestock

Generally, increase in livestock population is accompanied by high demands of livestock feeds. Feed demands by livestock can be met through efficient utilisation of natural resources or planted pastures and crops. Tropical fibrous pastures are converted into valuable animal products such as meat and milk (Khan *et al*. 2009) due to the ability of ruminants to use bacteria in the rumen to digest these feed materials. However, in Botswana, farmers who raise their livestock under communal grazing system do not have control over how pasture is used. The unsystematic use of natural pasture leads to problems like overgrazing and deterioration of vegetation, pasture quality thus resulting in a decrease in yields and digestibility (Khan *et al*., 2009). These problems were discussed by Masike and Urich (2008) as a tragedy of the commons. Basically, Masike and Urich (2008) were urging that lack of accountability in rangeland management in the communal system was a tragedy that has led to overgrazing and deterioration of pasture lands. Statistics Botswana (2015) reported an annual decrease in the pasture of 0.1% due to poor management practices. With a decrease in pasture land, alternative ways of feeding livestock are sought. Traditionally, crop residues from cereal and legumes are available after harvesting and are used to feed ruminant livestock. The advantage of using crop residues is that they are cheap and readily available during the summer period. However, their digestibility, especially cereal crop residues, is usually low due to high fibre and lignin content. Many methods have been used to overcome this low digestibility including grinding (Moreira *et al*., 2009), hydration (Ndlovu and Manyame, 2013), ammonisation/urea treatment (Kim and Lee, 2005), alkaline treatment (Chang and Holtzapple, 2000), acidic treatment (Kim *et al*.,2005) and ozonolysis (Nakamura *et al*., 2004).

These strategies have resulted in mixed outcomes. All these methods can be classified as either physical or chemical treatment but another method which is biological in nature has been reported in the literature. Hussain (2001); Khonga (2001) and Upadhyay and Sing (2010) show that growing mushroom on crop residues reduces lignin content thus improving digestibility if the remaining substrate is used as livestock feed. Mushrooms are fungi which secrete enzymes that help in extracting nutrients from the crop residues during its growth (Keller *et al*., 2003). Unlike enzymes from rumen bacteria which are unable to digest lignin, mushrooms provide an interesting strategy for bioconversion of fibrous and lignin-containing ruminant feeds. Mycelia of mushrooms embedded in the substrate (Ayala *et al*., 2011) are likely to increase the dry matter of digested component of the diet and resulting in an increase in the nutrient supply to the animal. This value addition to low quality cereal crop residues is expected to contribute to an increase in productivity and hence improved income (Khonga, 2003). However, cultivation of mushrooms in Botswana among both arable and horticulture farmers is not common. However, mushroom demand in the market is high (Beetz and Kustidia, 2004) hence its cultivation will add value to farming in two ways; by increasing income for the arable or horticulture enterprise and improving the nutritive value of the remaining substrate for feeding to livestock or as a soil conditioner.

2.1.1 Use of pasture as a ruminant feed

Grazing livestock rely on rangelands to meet their nutrient requirements for reproduction, growth and maintenance. For domesticated livestock, good grazing management is needed to control animal numbers as overpopulation may lead to overgrazing and low productivity. According to McDonald *et al*. (2011), pasture herbage provides low nutritive value for ruminants. For example, pasture grass provides 30 g/kg and up to 300 g/kg dry matter in mature herbage and fertilised young grass respectively. High levels of energy and crude protein are only realised when herbage is grazed while young. McDonald *et al*. (2011) also emphasised that the nutritive value of herbage often drops far below its optimum due to climate, soil and grazing management practised. According to Van Soest (2001), grazing management system employed may fail to ensure that pasture is grazed appropriately at growth stage when its nutritive value is high. Under tropical conditions where pasture is natural, nutritive value is often low with certain nutrients limiting the desired production levels.

Even though utilisation of natural pasture seems to be beneficial to most of Botswana farmers, it faces many challenges including overgrazing and poisonous plants. Natural pasture is also not adequate for high production due to reduced quality and quantity, this is exacerbated during the dry period.

Limitations associated with utilisation of pasture as an animal feed

Overgrazing and under-grazing

Report by McDonald *et al*. (2011) indicated that both under- and overgrazing of pastures may change their botanical composition and therefore its nutritive value. For example, in rotational grazing systems, pastures are foraged for short periods at a high stocking rate and grazing pressure (McDonald *et al*., 2011). Therefore, animals harvest most of the forage on offer, and the pastures are then rested for longer periods of recovery.

In Botswana overgrazing is one of the main challenges that most farmers face when using pasture, especially natural pasture, as a source of feed. It occurs when plants are exposed to intensive grazing for extended periods of time, or without sufficient recovery periods. Overgrazing reduces the usefulness, productivity, and biodiversity of the land and is one cause of desertification and erosion (Khan *et al*., 2009). Information by Van Soest (2001) shows that besides reducing productivity, overgrazing also causes the spread of poisonous plants and weeds. Overgrazing is indicated by the shortage of pasture for animals, which can be recognised by poor productivity and loss of body weight (Khan *et al*., 2009). This has serious implications for the health of livestock. Due to poor nutrition, female animals in poor body condition do not cycle as soon after calving, which can result in delayed breeding hence low livestock production (Bindari *et al*., 2013).

Approaches that can be applied to alleviate challenges that are associated with the use of natural pasture as an animal feed

To address problems associated with drought and feed shortages, scientists are investigating alternative feed sources from pasture. Feeding grains to ruminants is not favourable because human beings, a monogastric, use grains directly as food and ruminants would compete with humans. On the other hand, ruminants are characterized by their ability to convert low-quality roughage to products that are useful to human beings e.g. meat, milk, natural fibres, leather and manure. Ideally, ruminants should utilise roughages such as tree pods and crop residues due to the presence of micro-organism in their stomach.

2.1.2 Use of trees, pods and shrubs to complement pasture as an animal feed

According to Bakshi and Wadhwa (2004), shortage of grazing pasture, conventional feedstuffs for feeding livestock in developing countries like Botswana has forced farmers to use unconventional feed resources like forest tree leaves and pods. Fodder trees and shrubs provide high-quality nutrients for livestock in most tropical animal production systems. Moreover, their utilisation is increasing due to an increase in degradation of grazing areas (Bakshi and Wadhwa, 2004). Makkar (2001) stated that fodder tree leaves such as of *Grewia* spp. and *Leucaena leucocephala* have elevated protein amounts, soluble carbohydrates, minerals and show great potential as an alternate feed resource for livestock.

A report by FAO (2004) stated that shrubs and fodder trees are essential nutrient sources for ruminant animals, due to their capacity to eliminate feed shortages during winter or dry periods. During these times grassland growth is dormant due to dry to semi-arid climate like of Botswana. Leguminous and non-leguminous are suitable for feeding livestock since they provide large biomass due to their large numbers (Singh *et al*., 2003). Moreover, in some cases supplementation can be carried out through herbaceous and shrubby or tree legumes. To enhance utilisation of poor roughages (e.g. Sorghum stover), some farmers provide forage legumes as a supplement (Taylor and Bryant, 2007). According to Raihanatu *et al*. (2011), these legumes provide fermentable and escape/bypass protein and minerals and vitamins. Rodríguez *et al*. (2007) stated that digestible bypass protein is efficiently utilised and it is an important component in fast-growing beef cattle.

Research by Raihanatu *et al*. (2011) specified that protein amount of about 17% can be realised through utilisation of pods with high nutritive value. High nutritional value of trees and shrubs is realised during the rainy season than during the dry season. Examples are *Acacia nilotica*, which have edible pods, which become available during the dry season. The crude protein of pods ranges between 10-14% DM (Rubanza *et al*., 2005). *Acacia tortillis* is an excellent browse species for goats, having edible pods that are retained well into the dry season. Its protein content is about 167.85 g/kg (Mabeza *et al*., 2014).

Though trees/shrubs and pods have an advantage of supplementing grazing pasture, high palatability over other alternate feed sources, they have nutritional constraints like anti-nutritional factors which hinder animal performance.

2.1.3 Utilisation of crop residues as animal feed

The utilisation of crop residues as feed has been the subject of intense research and development worldwide. Crop residues are important for livestock feed and nutrient cycling among many other functions on smallholder farming systems in the tropics. According to Reddy *et al*. (2003), crop residues have the potential for lessening some of the feed shortages and nutritional deficiencies experienced in the dry season on smallholder farms respectively. In Botswana, different types of crop residues can be used as fodder for ruminants. They include cereal and legume crop residues. In many cases, crop residues are left in farms after harvesting and are being used in arable farming systems to promote soil fertility and erosion control (Lal, 2010).

Due to high availability in the dry season, crop residues have the capacity to complement the feeding natural pastures (Madibela and Lekgari, 2005), however, they have a low content of protein, minerals and vitamins. The quality of crop residues is affected by seasonal climatic changes and there is a need to evaluate them for use as fodder for ruminants in the dry season (Anandan *et al*., 2010). Unlike other feed resources that may be used on smallholder farms, crop residues have many advantages in that they are readily available on the farm and can be used for other purposes, thus effectively linking crop-livestock system.

Mahesh and Mohini (2013) stated that the capacity of field crops to be available during ploughing seasons enables them to produce crop residues in large quantities of biomass, which can be used for animal feeding. According to McCann (2001), in Southern Africa, crop residues derived from maize are abundant since maize is the main food crop accounting for 50–90% of the population's caloric intake. The yield of crop residues is affected by factors which normally affect the yield of crops (Raihanatu *et al*., 2011). Another important factor affecting the quantity of residue available for feeding, is that animals graze selectively, usually utilizing only certain parts of a plant or specific fractions of crop residues (McDonald *et al*., 2011). Other limitations accompanying utilisation of crop residues are trampling. When grazing residues, trampling contributes to the loss of edible material.

Although livestock use of crop residues is quite effective in reducing feed costs, their poor nutritional value and anti-nutrients are of great concern and may affect livestock performance.

Quality and quantity of crop residues

According to Shreck *et al*. (2011), most crop residues are low in protein and phosphorus, high in fibre and lignin. As a result, digestion is slow, the rate of passage is low and voluntary intake is limited. For example, the intake of sorghum stover is 43% less than that of hay, however, intake may be increased to about 20% by chopping the residue (Raihanatu *et al*., 2011).

To preserve the quality of crop residues through processing, extra labour is required and these increase the cost of production. Other problems experienced when feeding crop residues are bloating (Van Soest, 2001). Many crops are prone to regrowth and the young shoots cause prussic acid poisoning due to the presence of cyanogenic glycosides for example; Sorghum (Raihanatu *et al*., 2011). Some crop residues (soybeans hulls) produce trypsin inhibitors and solanine that hinder utilisation of proteins in diets (Gertenbach and Dugmore, 2004). Cauliflower residues block the uptake of iodine and when animals graze these residues for a long period, iodine deficiency symptoms occur (e.g. abortions and death of young animals) (Shreck *et al*., 2011).

The quantity of available crop residues is affected by all the factors that normally affect the yield of the grain crop. Another important factor affecting the quantity of residue available for feeding is that animal grazes selectively, utilizing only certain parts of crop residues, (Raihanatu *et al*., 2011). When grazing residues, crushing contributes to the loss of edible material. According to Shreck *et al*. (2011) physical processing of crop residues improves their intake by animals, however, animal performance might reduce as animals are forced to consume low quality material. When feeding crop residues to animals one should take into consideration that removal of residues from a field removes future soil nutrients, which must be replenished by organic and inorganic fertilisers. Animal grazing *in situ* assists in the breakdown of residues into easily decomposed components, thus returning of nutrients to the soil through manure dropped during grazing. The digestibility and palatability of crop residues could be improved through processing of crop residues. However, animal manure should be applied on the fields to improve the quality of the residues (Raihanatu *et al*., 2011). This interdependence between crops and livestock systems is suited for small-scale farmers as it reduced cost and increase overall productivity.

Cellulose is the most abundant structural carbohydrates (Isroi *et al*., 2011) followed by starch, in plants cell walls, their importance in ruminants' diets cannot be ignored. They are the primary sources of carbon in ruminants' diets, which is needed for microbial protein synthesis. Conversely, cellulose is bound to hemicellulose and lignin through the lignocellulosic complex (Mahesh and Mohini, 2013). Therefore, lignin should be removed in lignocellulosic complexes before feeding residues to livestock. Many methods have been investigated and these are reviewed in the following sections.

Pre-treatment processes employed for the conversion of lignocellulosic biomass into value-added products

Harmsen *et al*. (2010) described pre-treatment as the alteration of biomass, so that (enzymatic) hydrolysis of cellulose and hemicellulose can be achieved more rapidly and with greater yields. The overall goals of pre-treatment methods are to remove lignin and disrupt the crystalline structure of cellulose. There are physical, chemical and biological processes of pre-treating lignocellulosic biomass into value-added products for ruminant utilisation. These are reviewed below.

Physical processes of pre-treating lignocellulosic forages

Physical processing methods include grinding and blending crop residues, (Taylor and Bryant 2007). According to Harmsen *et al*. (2010), physical processes of removing lignocellulosic biomass are aimed at reducing the particle size of the feedstuff hence increasing the surface/volume ratio. This leads to more feed intake as there is a high passage rate of the feed due to a high digestibility since feed particles are exposed to enzymatic and microbial degradation (McDonald *et al*., 2011).

Blending

Blending is a process in which all feed nutrients in a crop residue are proportioned, processed and mixed into a uniform blend (Taylor and Bryant, 2007). Ibrahim and Olaloku (2000) stated that the quality and the palatability of crop residues and low-quality hay can be improved with the addition of other by-products from the sugar industry to maintain livestock performance. This replaces more expensive forage resources during times of forage shortage. Blending can be done by both small and large-scale farmers as it involves mixing high quality and low-quality feeds together in order to make a balanced ration. With this technology, the product can be fed as a sole source of nutrients. According to Taylor and Bryant (2007), blending ensures an adequate supply of balanced nutrients to the animal, controls the ratio of concentrate to roughage, helps in improving utilisation of low-grade fibrous crop residues and reduces feed wastage and feeding cost. The system also promotes feed intake and avoids refusal of the unpalatable portions of feedstuffs (Taylor and Bryant 2007). Such rations also reduce eating and rumination time and increase resting time.

Grinding or Chopping

Grinding is the most commonly used physical treatment of crop residues. According to Moreira *et al*. (2009), grinding reduces particle size and increases the surface area of the straws exposed to rumen microbial action. Gertenbach and Dugmore (2004) stated that processing of crop residues through milling increases the biomass ingested by the animal, as they are even forced to eat the lower quality material leading to reduced animal performance. Crop residues have the disadvantage of being extremely bulky, making costs of transportation high. One advantage of grinding or fine chopping is to reduce the bulk of the materials (Taylor and Bryant, 2007).

However, the processing of substrates through physical methods to improve their digestibility is accompanied by a rise in production costs. For example, purchasing hammer mill and fuel for grinding crop residues. On the other hand, using chemical methods seems to be a drawback because of the cost involved in the pre-treatment process, which are very high. Another important concern while using chemicals is the environmental concerns that are related to chemicals especially ammonia. Since ammonia is toxic to the environment, suitable strategies have to be maintained to prevent its escape into the environment. This additional feature also increases the cost of the process. After chopping, adding additives such as molasses and/or urea can also be used to improve intake and quality of crop residues. Crop residues are only available after the harvesting of crops and feeds for livestock should be made available even during periods of no growing crops.

Chemical processes of pre-treating lignocellulosic biomass

Chemical methods include treatment under acidic conditions, treatment under alkaline conditions, pre-treatment with ammonia, hydrogen peroxide and ozonolysis. Biological treatments include pre-treatment of biomass with fungal and bacterial strains for cellulose hydrolysis (Hasunuma *et al*., 2013).

Treatment with ammonia

Kim *et al*. (2003) stated that treatment with ammonia involves putting crop residues in aqueous ammonia treatment at elevated temperatures of 75°C. The process adequately reduces lignin content and removes some hemicellulose, while cellulose is also broken down. This was supported by (Kim and Lee, 2005) who stated that incubation in the presence of ammonia leads to hydrolysis of hemicellulose and destruction of lignin by ammonolysis. Mittal *et al*. (2011) emphasised that in this process cellulose forms a complex with ammonia resulting in breaking of hydrogen bonds in cellulose. Therefore, the method leads to the loss of crystallinity of cellulose making cellulose accessible to enzymatic hydrolysis and microbial degradation in the reticulorumen.

Kim and Lee (2005) also evaluated a method in which crop residues are soaked in ammonia under low temperatures of 30-70 °C. The results were that soaking in aqueous ammonia at low temperature removes lignin efficiently in the raw material by minimizing the interaction with hemicellulose. This method results in an increase of surface area, pore size and reserved hemicellulose/cellulose which can then be hydrolysed to fermentable sugars by an animal. Pre-treatment with ammonia has been shown to be an efficient method for biomasses with low lignin content, yielding (80-90 %) of reducing sugars (Bradshaw *et al*., 2007).

Pre-treatment of crop residues under alkaline conditions

In the alkaline treatment, crop residues are treated with alkalis such as potassium, calcium and ammonium hydroxides at normal temperature and pressures. A report by Chang and Holtzapple (2000) have validated that the process removes acetyl and uronic acid groups present on hemicellulose and thus enhances the accessibility to the rumen microbes that degrade hemicellulose. According to Sun and Cheng (2002), the ester linkages that connect the xylan and hemicellulose in the residues are broken down through hydrolysis. When using calcium hydroxide or sodium hydroxide, the reaction is mild thus preventing condensation of lignin and its excessive removal (Sharma *et al*., 2012). In this mild reaction, there is minimal degradation of sugars and the process is usually applied to residues with low lignin levels like grasses (Chang and Holtzapple, 2000).

Treatment by ozonolysis

Nakamura *et al*. (2004) described ozonolysis process as a process in which crop residues are treated with ozone, which causes the destruction of lignin through degradation of aromatic rings structures, while hemicellulose and cellulose are not affected at all. Sun and Cheng (2002) supported that this process can be employed to interrupt the structure of lignocellulosic materials such as wheat straw, pine and maize stover. This technique was also investigated by Garcı´a-Cubero *et al*. (2009) on wheat straw and rye with the intention of increasing enzymatic hydrolysis extent of potentially fermentable sugars. The result of the experiment was that enzymatic hydrolysis yields of up to 88.6 and 57 % compared to 29 and 16 % in non-ozonised wheat and rye straw, respectively. Ozonolysis is affected by the level of moisture and type of crop residues. According to Miura *et al*. (2012), when the moisture content of crop residues reaches more than 40 %, ozone consumption decrease, resulting in less delignification.

According to Barros Rda *et al*. (2013), ozonolysis alone has been shown to be ineffective for removal of lignin and yields of reducing sugars. However, using ozonolysis and other treatments like milling and pelleting increase lignin removal. Barros Rda *et al*. (2013) reported 90% of sugar yields when combining ozonolysis and milling.

Treatment under acidic conditions

This process involves the treatment of lignocellulosic biomass with diverse acids such as sulphuric acid, oxalic acid and peracetic acid, respectively. Kim *et al*. (2005) consider the use of diluted acid treatments to be very effective and cheap since low concentrations of acids are used. Acid treatment method can also be carried out in high acid concentrations and at high and low temperatures. The method involves spraying sulphuric acid on raw crop residues which are later incubated at 160–220°C for few a minutes (Kim *et al*., 2005). These high temperatures hydrolyse hemicellulose, discharging monomeric sugars and soluble oligomers from the cell wall matrix. Hemicellulose removal improves enzymatic digestibility of cellulose by ruminants (Kim *et al*., 2005). One disadvantage of this technique is that when using dilute acids, only hemicellulose is hydrolysed, whereas cellulose and lignin are unaffected (Kim *et al*., 2005).

Treatment by hydrogen peroxide

Hydrogen peroxide is the most commonly employed oxidizing agent that is used to reduce lignin levels in crop residues (Hammel *et al*., 2002). A study by Hammel *et al*. (2002) also indicated that dissolution of 50 % of lignin and most of the hemicellulose has been achieved in a solution of hydrogen peroxide at a temperature of 30°C. This achievement is due to the production of hydroxyl radicals, which degrade lignin (Hammel *et al*., 2002) and leads to exposure of hemicellulose and cellulose causing elevated microbial and enzymatic hydrolysis (Hammel *et al*., 2002). Yu *et al*. (2009) achieved dissolution of about 90-95% when combining hydrogen peroxide (chemical method) and using *Pleurotus ostreatus* (a biological method involving the use of mushroom species) to treat rice hull. The combined pre-treatment led to significant increases in the lignin degradation.

Since the conversion of lignocellulosic biomass using methods discussed above requires large inputs of resources and cause pollution, alternative methods for lignin degradation should be put into place. They include biological treatment of crop residues which is considered as an efficient, eco-friendly and cheap alternative (Wan and Li, 2011).

Biological pre-treatment of crop residues for animal feeding

Biological treatments include pre-treatment of biomass with fungal and bacterial strains for cellulose hydrolysis (Hasunuma *et al*., 2013). The biological pre-treatment process involves using cellulolytic microorganisms which synthesize potent cellulolytic enzymes during hydrolysis (Wan and Li, 2011). In most cases, biological treatment involves utilisation of filamentous fungi, which survive in diverse environments such as living plants and lignified waste materials. Okano *et al*. (2005) stated that white-rot fungi are effective microorganisms for the treatment of waste products such as wheat straw, Bermuda grass and different cereal crop stover. Rasmussen *et al*. (2010) explained that fungi in *Ascomycota* and *Basidiomycota* are capable of degrading cellulose, hemicellulose and lignin and are considered as major degraders of woods in forest ecosystems. In a study by Saritha *et al*. (2012) hardwood and softwood were treated using *Streptomyces griseus* isolated from leaf litter and solubilisation of lignin was reported on wood substrates. Sun and Cheng (2002) detailed that the merits of biological pre-treatments are their low energy requirement and mild operating conditions. However, the rate of biological hydrolysis is usually very low, hence it takes a long time to degrade cellulose and lignin in residues.

Feasibility and economic assessment of pre-treatment methods

Harmsen *et al*. (2010) emphasised that the choice of a pre-treatment method should not only be grounded only on its potential yield but also on important parameters such as its economic assessment and environmental impact. Chaturvedi and Verma (2013) stated that there is no pre-treatment technique which offers 100% conversion of lignocellulosic biomass to fermentable sugars. This is because some of the biomass is lost during the process of conversion. The common goal of pre-treatment techniques is to improve the digestibility of lignocellulosic biomaterials as they have different effects on lignin, cellulose and hemicellulose portions (Harmsen *et al*., 2010). Chemical pre-treatment methods such as concentrated acid hydrolysis and dilute acid pre-treatment (Mosier *et al*., 2005) are reported to be cost-effective when compared to physical methods. Moreover, they are mostly applied in industries for the production of ethanol, not small-scale farmers (Harmsen *et al*., 2010). On the other hand, physical methods such as grinding/ milling aim at reducing the particle size of lignified crop residue, making the material to be handled easier and increase surface/volume ratio (Moreira *et al*., 2009). However, they are normally accompanied by high energy, capital costs and normally carried out by large-scale farmers in Botswana. Chemical pre-treatment method like ozonolysis requires a high amount of (ozone) O_3, which is expensive, so the pre-treatment method is less cost-effective than others discussed (Chaturvedi and Verma, 2013). Pre-treatment under strongly acidic conditions requires materials which can resist corrosion and this increases its capital costs. There is also an issue of safety, health and environmental risks. Such methods are not practicable to small-scale farmers of Botswana since they have limited resources.

Throughout the discussion on the utilisation of natural pasture and crop residues, limitations such as poor nutritional value, uncontrolled grazing, poisonous plants and low digestibility have been highlighted. It is the hypothesis of this project that challenges that are associated with feeding crop residues (which are abundant feed source after harvesting) can be alleviated through alternative treatment methods. This can be achieved by incorporation of oyster mushroom (*Pleurotus ostreatus*) production into cereal production system. Mushroom production generates a virtually inexhaustible supply of a product called spent mushroom substrate (SMS). This unutilised substrate and the mushroom mycelium left after harvesting of mushrooms represent an unutilised resource for animal feed.

Spent oyster mushroom substrates provide quality feed for livestock as the lignin and cellulose are degraded by the mycelium in oyster mushroom compared to non-spent substrates (Xu *et* *al*., 2010). Low-quality livestock fodder in Botswana can be mitigated by using SMS which is assumed to have higher crude protein and lower crude fibre contents compared with the original substrate. Xu *et al*. (2010) reported that supplementation with SMS improved weight gains and feed efficiency in ruminants mainly due to more feed intake as animal's retention time of spent substrates is reduced hence more intake of nutrients.

2.2 Oyster mushroom production

With low and erratic rainfalls recently experienced in Botswana due to global warming, constant and reliable fodder production will always remain a challenge. It is of great interest to explore the incorporation of oyster mushroom production into crop production to produce agricultural by-products like mushroom substrate which can then be fed to ruminant animals. The use of this agricultural product could reduce feeding costs during the dry seasons and lessen environmental concerns of pollution (Park *et al.*, 2012).

According to Upadhyay and Sing (2010), production of oyster mushrooms (*Pleurotus* spp.) started in Germany in 1917. These mushrooms were cultivated on tree stumps and wood logs. As time went on, growing technologies improved and differed from region to region in around the world (FAO, 1990). Just like other types of mushrooms, *Pleurotus* spp. can be grown on different crop residues with the use of different substrate containers such as trays, plastic containers and bottles (Mamiro *et al*., 2014). Presently, in many developing countries like Botswana, oyster mushrooms are produced by using plastic bags as substrate containers with substrates being maize, sorghum and millet stalk (Khonga, 2001). According to Oei (2003), plastic bags are used for fructification and they are made from polyethylene if pasteurized substrates are used. Mushworld (2004) stated that for a sterilized substrate, polypropylene or polyvinyl chloride plastic bags are used.

Two phases are involved in the mushroom production. The first phase is spawn making which is the aseptic culture of mushroom mycelium and secondly; mushroom growing which is the growth and development of mycelium under protected but non-aseptic conditions to produce mushrooms. According to Hussain (2001), oyster mushroom production is an economic and efficient possible biotechnology to convert agricultural and industrial wastes into high-quality protein foods. Agricultural and industrial wastes are a source of nutrients for the mushroom growth as they are composed of cellulose, hemicellulose, and lignin (Rodriguez *et al*., 2008) which are needed for mycelium growth. Orts *et al*. (2008); Saber *et al*. (2010) indicated that wheat and rice straws, wheat bran, hardwood chips, sugarcane bagasse, cotton seed hulls, corn cobs, rice, and sawdust are the most used waste for production of edible mushroom. Wheat straw is characterised by high plant nutrients which accounts for 35–40 % Nitrogen, 10–15 % Phosphorus, and 80–90 % Sodium (Davari *et al*., 2012). These nutrients are responsible for the high nutritive value of mushrooms, spawn running and the size of mushroom (Peng *et al*., 2000). Liu *et al*. (2010) analysed oyster mushroom and found out high amounts of polysaccharides (including glucans), fatty acids, and vitamins, antioxidants, anti-angiogenetic properties against tumours (Shenbhgaraman *et al*., 2012) and intracellular β-glucan molecules which keep the immune system healthy (Carbonero *et al*., 2006).

However, the nutritive value of mushrooms is also influenced by factors which influence their productivity. Jafarpour and Eghbalsaeed (2012) emphasised that mushroom productivity is influenced mainly by the type, amount and nutritional quality of the substrate used. These factors also are responsible for the interaction of mycelium and cellulose, hemicellulose and lignin.

2.2.1 Agricultural waste materials used in the cultivation of oyster mushrooms

Sharma *et al*. (2013) stated that cultivation of oyster mushroom (*Pleurotus ostreatus*) has increased enormously through the world because of their abilities to propagate at a varied range of temperature and utilizing various agroforestry by-products and agro-based residues. This was supported by Gregori *et al*. (2007) who stated that oyster mushrooms can even use weeds to produce food, feed, enzymes and medicinal compounds, or for waste degradation and detoxification.

Table 2.2.1. Summary of various substrates that can be used to cultivate mushrooms.

Type of substrate	Reference
Pearl millet straw and chaff, cowpea crop residues, maize cobs and straw	Horn (2004)
Cassava peel, cotton waste, palm oil chaff, and vegetable*)*	Amuneke *et al*. (2011)
Seagrass, paddy straw, wheat straw, cotton waste	Ashraf *et al.* (2013); Mamimuthu and Rajendran, (2015)
Wheat stubble, date palm leaf and potato dextrose agar	Kabirifard *et al.* (2012)
Rice straw, sawdust, corn cobs, rice bran, wheat bran, beet pulp, and bean curd	Park *et al.* (2012)
Sawdust of *Leucaena leucocephala*	Pant *et al*. (2006)

2.2.2 Benefits of mushroom production

The benefits of mushroom production are the generation of millions of tons of residue referred to as spent mushroom substrates (SMS) which remains after the mushroom crop has been harvested. These are renewable agricultural residues which represent an abundant, inexpensive and readily available source of renewable lignocellulosic biomass as reported by Foluke *et al*. (2014).

2.2.2.1 Spent mushroom substrates

A spent mushroom substrate is a by-product produced from mushroom production and is among the agricultural by-products of interest (Bae *et al*., 2006). A study by Paredes *et al*. (2009), indicated that it is feasible to use these kinds of waste to produce animal feed as they are rich in minerals such as iron and silicon. According to Bae *et al*. (2006), spent mushroom substrates are a nutrient-rich organic by-product of the mushroom industry. Xu *et al*. (2010) stated that *Pleurotus* spp. are efficient lignin degraders, which can grow on different agricultural wastes with broad adaptability to varied agro-climatic conditions and require a short growth time in comparison to other edible mushrooms.

Ayala *et al*. (2011) stated that mushroom substrates have fungi which degrade lignocellulose, resulting in improved feed nutritional value. Kim *et al*. (2010) suggested that mushroom spent substrate can be ensiled and be used as an appropriate forage source in maintenance rations for ruminants. They can be easily digested by ruminants' due to the enzymatic conversion processes during mushroom cultivation. Zhu *et al*. (2012) stated that, crude protein, fat content and ash content of the spent substrates increase with time but not the cell wall components, with the most remarkable change of composition in the spent straw substrate is the reduction of hemicelluloses by 17 %, cellulose by 15 %, lignin by 4 % while gossypol reduced by 60 % This may be due to the fact that most of the cell wall components are degraded by enzymes secreted during mycelium growth and mushroom production. This would directly increase the *in vitro* dry matter digestibility (IVDMD) of SMS of oyster mushroom as a ruminant feed. Furthermore, SMS also generates most of the polysaccharides, vitamins and some trace elements such as Fe, Ca, Zn and Mg, which are valuable for animals (Medina *et al*., 2009).

With these abundant nutrients in SMS, they can be used as a potential source of feed for small stock.

Benefits of using spent mushroom substrates (SMS) in ruminants

The use of SMS of oyster mushroom, as feed ingredient has several advantages. It is a local resource that reduces cost. Its utilization will help to solve the environmental issue that could result from the accumulation of SMS after mushroom production. Farmers can benefit from feeding SMS of oyster mushroom as fodder since it is more digestible than the raw crop residues from arable farming (Rinker *et al*., 2004). They can also benefit by giving the crop harvest residues to mushroom producers and get the more valuable spent mushroom substrates (Siddhant and Singh, 2009) in return, to feed their ruminants. Mushroom farmers can sell the spent substrate to ruminant livestock farmers. This will enhance the efficiency of the crop-livestock system, contribute towards mitigating strategies for climate change by reducing carbon costs.

Improved nutritive value

At the end of several mushroom harvests, the growing material is considered spent i.e. spent mushroom substrate (SMS). SMS contains enough digestible nutrition, primarily decomposed by mushroom, to be fed livestock (Rinker *et al.,* 2004). Proximate analysis of Oyster mushroom wheat spent substrate by Foluke *et al*., (2014) revealed % Crude Protein 7.88, Crude Fiber 29.57, Ether Extract 1.71, Ash 9.92 and Nitrogen free extract 42.85. Xu *et al.* (2010) evaluated the nutritive value of total mixed ration (TMR) silages that were made from ensiling of TMR containing spent mushroom substrate (SMS) and discovered that increasing concentration of SMS in the ration tended to decrease content of total volatile fatty acids in rumen fluid but increased the ratio of acetate to propionate and rumen pH. The study further suggested that an SMS level of 6.5% in the diet DM can be recommended for silage-based TMR.

The dry matter and composition of cottonseed hull substrate used for *P. ostreatus* cultivation were tested at different growth stages by Li *et al.* (2001) and they discovered that as protein, cellulose, hemicellulose and lignin were used by *P. ostreatus*, the compositional profiles of the substrate were changed greatly, but their rates of change varied at different growing stages. The increase of protein content and the reduction of lignocellulose content contributed to the increase in the dry matter digestibility of the spent substrate, making it possibly acceptable as a potential ruminant feed. Darwich *et al*. (2012) reported that a single *P. ostreatus* treatment of maize stalks gave lower protein content when compared to simultaneously double-treated with *P. ostreatus* and *Saccharomyces cerevisiae* while cellulose, hemicellulose and lignin showed a gradual decrease with increasing incubation time of the fermentation process. The nutritive value of rice straw treated with three different *Pleurotus* spp; *P. ostreatus*, *P. pulmonarius* and *P. tuber-regium* had higher crude protein but lower crude fibre cellulose, neutral detergent fibre, acid detergent fibre and acid detergent lignin compared to untreated control (Akinfemi and Ogunwole, 2012; Thi Huyen *et al*., 2019). They concluded that straw treated with *Pleurotus* spp. had improved its nutritional value as livestock feed.

A study conducted by Adamovic *et al*. (2007) with the increase of spent *Pleurotus ostreatus* mushroom substrate ratio in silage (from 10 to 30%), observed that values for NDF, ADF, hemicellulose, cellulose and lignin, linearly increased. The use of this kind of silage in smaller quantities (up to 10% of dry matter in diets for cows and fattening bulls) could be reasonable in diets with low ADF and NDF content, as well as for the cattle, with lower genetic potential, under extensive conditions of nutrition (Adamovic *et al*., 2007).

Analyzed effects of mushroom cultivation on nutritional value and dynamics of rumen degradability of cottonseed hull and SMS by Pan *et al*. (2012), proved that nutritional value and effective rumen degradability of the SMS improved by mushroom cultivation on cottonseed hulls were also fit for use as feed for ruminants.

Table 2.2.2.1. Minerals content of maize stalks (mg/kg)

Feed ingredient	Ca	P	Mn	Zn	Cu	Mg	Fe
Maize stalks raw	800	370	462	41.80	26.20	3900	517.80
Maize stalks after fermentation	830	375	502	42.64	27.21	4200	523.71

Source: Darwich *et al*. (2012)

From Table 2.2.2.1, it is evident that the mineral content of maize stalk increased after fermentation using *Pleurotus ostreatus* and *Saccharomyces cerevisiae*.

Improved animal performance

Kim *et al*. (2011), discovered that growth performance (average daily gain) was higher (0.68 kg) in strains-fermented sawdust-based mushroom spent substrate supplement (fOMSS) group than (0.63 kg) and 0.64 kg of fermented concentrates and direct-fed microbes (DFM) respectively. The strains used in the fOMSS diet were various combinations of lactic acid bacteria (*Pediococcus acidilactia* CAM1, *Pediococcus acidilactia* Pa175 and *Lactobacillus plantarum* Lp177), yeast (*Saccharomyces boulardii* Sb796) and Bacillus (*B. polymyxa* T-1 and *B. subtilis* T-4). This indicated that fOMSS has the beneficial effects of an alternative to antibiotics for a growth enhancer in dairy calves.

Kim *et al.* (2012) reported an increase in total voluntary dry matter intake (DMI) of rice straw of 7 to 15% with dietary supplementation of microbially-fermented spent mushroom substrates (MFSMS) resulting in an increased in live weight gain. Investigated effects of supplementation of spent mushroom substrates (SMS) on rumen fermentation and blood metabolites in Hanwoo steers by (Oh *et al*.,2010) indicated that SMS of *Pleurotus* spp. could be used as a forage source to replace 40% of rice straw without any negative effects on rumen fermentation and blood metabolites in Hanwoo steers.

Reduction in methane production from fungal treated substrates

Methane emissions from livestock are a significant contributor to greenhouse gas emissions and have become a focus of research activities, especially in countries where agriculture is a major economic sector like in Botswana. Moreover, methane emissions are responsible for 4 to 13% loss of energy by ruminants (Kristensen *et al.*, 2011). This process is referred to as enteric methane and released into the atmosphere the methane gas. Methane production in the rumen occurs because of the presence of a group of microorganisms called methanogens that are normally found in the reticulorumen and large intestine of ruminant livestock (Morgavi *et al.*, 2010).

Morgavi *et al*. (2010) described the pathway by which methane is produced by ruminant animals. According to these authors, the methanogens microbes reduce carbon dioxide (CO_2) to CH_4 in the rumen through utilisation of hydrogen (H_2) which is an abundant product of feeds fermentation by bacteria, protozoa and fungi. According to McGinn *et al*. (2004), the amount of H_2 in the rumen depends on the type of volatile fatty acids (VFAs) which are produced during feed fermentation. McGinn *et al*. (2004) also indicated that acetate formation produces twice the amount of H_2 when compared to butyrate, hence more methane production as dietary carbohydrates influence the relative proportions of VFAs. According to Boadi *et al*. (2004), methane production of ruminal microflora is high during the fermentation of structural carbohydrates compared to that of non-structural carbohydrates.

Boadi and Wittenberg (2002) stated that most of the dietary factors that result in a reduction in feed residence time will always result in low methane emissions. Factors such as diets rich in starch and very low in fibre will favour propionate production hence lower CH_4 emissions. This was supported by McAllister and Newbold (2008) who emphasised that diets that produce a low ratio of acetate to propionate will result in a decrease in CH_4 production. This is because the diets restrict the formation of H_2 in the rumen hence less methanogenic activity to reduce

CO_2 to CH_4. The reason behind this is because microbial digestion of the feed is reduced and low acetic to propionic acid ratio is favoured (Hook *et al*., 2010).

According to Mahesh and Mohini (2013), low methane emissions have been recorded in diets containing cereal stover subjected to mushroom treatment. Methane levels of 200 ml/mg^{-1} from *in vitro* fermentation of peanut husk were reported by Akinfemi (2010). This value was significantly lower than the one (280 ml/mg^{-1}) reported in untreated husk fermented for 21 days *with Pleurotus ostreatus* (white rot fungus). This show that methane production from fungal treated wheat straw reduced significantly and this has the potential to contribute to environmental friendly ruminant diets. A study by Mahesh (2012) revealed a linear reduction in (%) methane from fungal treated wheat straws which had fewer fibre fractions (Neutral Detergent Fibre and Acid Detergent Fibre) than untreated straw. This might be due to less fibre being exposed to digestion in the rumen. Sallam *et al*. (2007) stated that this was probably due to indirect effect by fibre digestion leading to the lesser residency of feed particles in the rumen. It can be concluded that enteric methane emissions are highest when the animal is presented with poor quality forages such as crop residues.

Through fungal treatment of crop residues, benefits of feeding poor quality forages can be realised. This is because of an improvement in the forage quality with respect to cell wall digestion and overall enrichment in carbohydrates digestibility as well as increased DM intake. From the literature mentioned methane emission is significantly reduced when oyster mushroom spent substrates are used as animal feed compared to non-fungal treated straw. Even though studies have already established that SMS are useful, their inclusion level of incorporation in total mixed rations or complete diets has not been studied extensively. On the other hand, their inclusion in diets is influenced by the chemical attributes of the improved straw which has not been adequately studied. These will help to solve the problem of feed crisis in small farming system in developing countries like Botswana.

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CHAPTER THREE-STUDY ONE

3.0 Assessment of current uses and yields of crop residues by small-scale farmers in Kgatleng, Kweneng and Southern districts of Botswana

Abstract

A survey was conducted in August 2017 to assess the current uses and yields of crop residues by small-scale farmers in Kgatleng, Kweneng and Southern district of Botswana. Data were collected from 63 farmers with 22 of them from Kgatleng, 25 from Kweneng and 16 from Southern district. The results show that 41.3% of the farmers were females and 58.7% were males. About (65.1%) of the farmers were married while 15.9% were single and another 15.9% were widowed. About 42.1% were more than 65 years old. About 92.1% of farmers had formal education. Ninety-five point five percent (95.5%) of small-scale farmers owned the ploughing fields and 87.3% were committed to full-time farming. The average size of the fields for ploughing was 14.27 hectares (Ha). Crop residues were harvested and stored by 66.7% of farmers for later use, with 33.3% of farmers allowing their livestock to feed on crop residues *in-situ*. Crop residues were mostly fed to ruminants, with 50.8% of the livestock fed being cattle and goats. All classes of livestock constituting 98.4% were offered crop residues. Farmers had different perceptions of the quality of the cereal crop residues fed to livestock. About 14.3% perceived Sorghum crop residues to be of high quality when compared to other crop residues with 54.0% indicating that maize crop residues are of very low quality. Moreover, 98.4% of the farmers could not rate the value of millet crop residues. To improve nutritional quality and better utilisation of crop residues by livestock, 73.0% of the farmers added feed ingredients such as protein sources (cowpeas residues and lablab), minerals (salt and di-calcium phosphate) and concentrates (molasses and wheat bran). However, 27.0% of the farmers did nothing to improve the quality of residues which they perceived to be of poor quality. About

95.2% of the farmers were willing to employ treatment of crop residues through mushroom production as a strategy of improving quality while 4.8% only wanted to adopt mushroom production for income generation. Many farmers (85.8%) agreed to participate in the mushroom production project. It is recommended that farmers improve the quality of crop residues which are abundant for improved animal performance and productivity.

Keywords: Botswana, crop residues, current uses, small-scale farmers

3.1 Introduction

In Botswana, cereal crop residues are mainly from maize, sorghum and millet, while legume residues comprise groundnuts and cowpeas (FAO, 2004; Statistics Botswana, 2016). During cereal harvesting, which is done using a knife, the grain head is removed and transported to a homestead where threshing is done. The maize cobs, chaff and glumes for millet and sorghum are often burnt and not stored for livestock feeding. The standing stover in the field is left to be grazed and in the process trampled on by livestock (Madibela and Lekgari, 2005). Some farmers cut and store the crop residues for feeding livestock during the dry periods. However, some farmers may improve the nutritive value of the collected residues by adding other feed ingredients. The amount of crop residues harvested depends on the crop yields and harvest index (HI) or a ratio of residue to grain (Unkovich *et al*., 2010; Dai *et al*., 2016). This method can also be used to estimate crop residue yields in Botswana. Based on this method, crop residues in the three districts based on grain yield estimate from total harvested areas in 2014 (Statistics Botswana, 2016) were 153,860, 354,180 and 413,250 Metric tonnes for Kgatleng, Kweneng and Southern districts respectively. Alternatively, to estimate crop residues collected, a piece of land may be measured and crop residues collected from it also measured. There is limited information (Legodimo, 2013) on the utilisation of different cereal crop residues at the farm level by small-scale farmers. Therefore, the objective of this study was to assess the current uses of crop residues and yields in Kgatleng, Kweneng and Southern districts of Botswana.

3.2 Materials and methods

The survey was carried in August 2017. A structured questionnaire was developed (Appendix) and administered during a face to face interview. Participating farmers with 100 Km radius from BUAN were identified with the help of District Agricultural Coordinators (DACs) of the

Ministry of Agricultural Development and Food Security in Kgatleng (22), Kweneng (25), and Southern districts (16) respectively. The survey questionnaire was administered by visiting farmers from the following villages Oodi, Modipane and Malotwane in Kgatleng district; Medie, Kumakwane and Gabane in Kweneng district; and Metlojane and Metlobo in Southern district. The interviews were conducted either at the farm or at the homestead.

Estimation of the yield of maize, sorghum and millet stover per Ha was done by cutting and collecting all above ground stover from three replicate 10m x 10m plots per farm of maize, millet and sorghum respectively. Maize and sorghum residues were estimated in Gabane farms which were about 3 to 4 Km apart while millet crop residues were estimated in BUAN farm.

Figure 3.2.1. Collection and weighing of cut millet crop residues for estimation of yields.

3.3 Data analysis

Survey questionnaire data were analysed using qualitative statistics (means, frequencies standard deviations and Chi-Square. The IBM SPSS statistics (2013) version 22 was used.

3.4 Results

3.4.1 Biographic data of the respondents

Out of the 63 respondents, 41.3% and 58.7% were females and males, respectively. The age distribution of the farmers was a follows: 42.9% aged 65 years and above; 19% aged 61-65 years, and 38.1% were less than 60 years old. The education levels of the majority of respondents was primary school (57.1%) followed by secondary (11.1%) and tertiary (9.5%). Sixty farmers (95.2%) owned the fields while 3 (4.8%) rented the land.

3.4.2 Cereal crop residues and their disposal

The majority of farmers (66.7%) harvested and stored cereal crop residues for feeding animals while the minority (33.3 %) left the crop residues to be grazed *in-situ* by livestock.

Table 3.4.2. Disposal of Cereal Crop after Harvest

Method of Disposal	Frequency	Percent	Cumulative Percent
Harvested and stored for feeding animals	42	66.7	66.7
Left in field to be grazed by animals	21	33.3	100.0
Total	63	100.0	

On which animals' farmers fed with crop residues, most farmers (50.8%) fed both cattle and goats followed by cattle alone (22.2%), cattle, goats and sheep (14.3%) and goats alone (7.9) and cattle, goats and donkeys (4.8%).

Table 3.4.3. Species of Animals Fed with Cereal Crop Residues

Species	Frequency	Percent	Cumulative Percent
Cattle and goats	32	50.8	81.0
Cattle	14	22.2	22.2
Cattle, sheep and goats	9	14.3	95.2
Goats	5	7.9	30.2
Cattle, goats and donkeys	3	4.8	100.0
Total	63	100.0	

On animal age classes fed with residue, 98.4% of the farmers indicated that they fed residues to all age classes of animals and only 1.6% fed residues to calves only.

Table 3.4.4. Age class of animals fed with cereal crop residues

Class	Frequency	Percent	Cumulative Percent
All classes	62	98.4	98.4
Calves	1	1.6	100.0
Total	63	100.0	

On farmers' perception on the nutritional quality of maize crop residues 54.0%, 31.7%, 12.7 and 1.6% rated them as very low quality, low quality, did not know and high quality, respectively. On the other hand, the ratings for sorghum crop residues were 6.3%, 74.6%, 3.2%, 14.3% and 1.6% for very low quality, low quality, don't know, high quality and very high quality, respectively. However, for millet 1.6% and 98.4% of farmers rated them very low and don't know, respectively indicating that millet stover was not used as feed in the surveyed areas.

Table 3.4.5. Perceived quality of Maize, Sorghum and Millet crop residues by farmers

	Frequency			Percentage (%)		
Nutritional Value	Maize	Sorghum	Millet	Maize	Sorghum	Millet
Very Low Quality	34	4	1	54.0	6.3	1.6
Low Quality	20	47	-	31.7	74.6	-
I Don't Know	8	2	62	12.7	3.2	98.4
High Quality	1	9	-	1.6	14.3	-
Very high quality	-	1	-	-	1.6	-
Total	63	63	63	100	100	100

In order to improve nutritional quality of crop residues the majority of farmers (15.9%) added salt only, 11.1% added molasses, wheat bran, and salt; 9.5% added molasses and salt, 7.9% added molasses, Di-calcium and salt 6.3% added lablab and salt, 6.3% added cowpeas stover and molasses, 3.2% added lablab alone and 1.6% each added wheat bran and salt and calf and goat meal, and 27% added nothing to the stover.

Table 3.4.6. Measures Taken to Improve Quality of Cereal Crop Residues

Supplement added	Frequency	Percent	Cumulative Percent
Salt	10	15.9	15.9
Molasses	3	4.8	20.6
Molasses and salt	6	9.5	30.2
Molasses and beef finisher	1	1.6	31.7
Molasses, wheat bran, and salt	7	11.1	42.9
Molasses, wheat bran, beef grower and salt	1	1.6	44.4
Molasses, Di-calcium and Salt	5	7.9	52.4
Molasses, lablab and salt	1	1.6	54.0
Lablab	2	3.2	57.1
Lablab and salt	4	6.3	63.5
Cowpeas stover and molasses	4	6.3	69.8
Wheat bran and salt	1	1.6	71.4
Calf and goat meal	1	1.6	73.0
Nothing done	17	27.0	100.0
Total	63	100.0	

On willingness to participate in using crop residues for mushroom production in order to improve crop residue utilisation by livestock 95.2% of the farmers were willing and only 4.8% were willing to grow mushrooms only for income generation. However, when interviewed on how likely they were to take part in bio-conversation of cereal crop residues through mushroom production, 68.3% and 17.5% were very likely and likely while 11.1% and 3.2% were undecided and unlikely, respectively.

3.4.3. Estimation of yield of crop residues on farmer's fields

From 10m x10m plots replicated three times, average maize and sorghum yields were 12.13 and 12.00 kg/100 m^2 respectively in Gabane area. Estimated millet yield recorded at BUAN farm was 99.07 kg/100 m^2. When estimated per hectare, maize, sorghum and millet yields would be 1213 kg, 1200 kg and 9907 kg respectively. Recorded millet stover yields were high compared to that of the other stovers and this could be due to high planting density as it was used as forage for farm animals. Moreover, the soil fertility of BUAN farm could have been high as fertilisers are applied compared to that of Gabane farms where farmers do not test and apply fertilisers before they plough.

3.5 Discussion

In a study conducted by Mahabile (2006) on determinants of herd productivity in Botswana, most of Botswana farmers were old and males. This observation was noted to have a negative impact on the dairy industry of the country. In the present study, the same observations were made and this could have an adverse effect on the production of crops and food security. Most of the farmers in the present study were literate (92.1%) and this was a positive finding as education is needed for development of crop production systems in the country. Similar findings were reported by Mahabile and deWaal (2010). The structure of crop production in Botswana which is represented by old aged individuals which are mostly males implies that more experience is needed in farming. However, it may delay the adoption of new technology that is involved in the growing of crops. Youth should be encouraged to be involved in farming activities on a full-time basis as this will help the country to be food secure.

Devi *et al*. (2017) stated that crop residues are offered to domestic ruminant animals in forms of traditional stubble grazing and this was observed in the present study as 33.3% of the farmers allowed their livestock to graze on crop residues *in-situ*. Additionally, crop residues could be prepared and mixed with nitrogen compounds to improve their nutrition and palatability (Devi *et al*., 2017). This was also observed in the current study as 66.7% of the farmers harvested and stored them for use during dry periods. During the dry seasons, they mixed them with other feedstuffs such as salt, cowpeas residues, molasses, wheat bran, lablab and di-calcium phosphate. This was done to enhance the quality of maize which was perceived to be poor by 54% of farmers while the quality of millet was not known by 98.4% of the farmers. Mosimanyana and Kiflewahid (2006) supplemented crop residues with high protein legumes such as cowpeas or lablab and locally produced milling by-products (Moroko). Most of the farmers practised feeding system based on natural pasture during summer months while crop residues were fed during winter when feed shortage was high. Importance of proper storage of crop residues after harvesting must be emphasised in order to preserve the quality of residues. Other strategies of improving quality of crop residues such as mushroom cultivation (Kumar *et al*., 2016) have been employed elsewhere and in the present study 85.8% and 98.4% of farmers agreed to participate in this study and adopt this strategy respectively. The fact that most farmers feed crop residues to adult ruminants shows that most understand the class of animals which will utilise the crop residues better. Calves have less developed rumen and would not utilise fibrous crop residues efficiently.

The current yields of maize and sorghum crop residues in the present study were low when compared to that reported by (Mosienyane, 1983). The author estimated maize, sorghum and millet yields of 3.73,3.50 and 3.05 tonnes/ha respectively. Due to climate change, rainfall patterns change hence directly or indirectly affecting crop yields. In the 80's, Botswana experienced high and reliable rainfall compared to in recent times, hence high yields of crop residues. Mosienyane (1983) stated that high quality and quantity of cereal crop residues could be achieved when crop residues are harvested early. Estimation of crop residue yields can serve as an indicator of quantity and location of an otherwise disused feed resource (Mosimanyana and Kiflewahid, 2006).

3.6 Conclusions

Farmers committed to the full time and part time growing of crops were characterised by the similar production of sorghum, millet and maize cereal crops with maize being the most grown crop in their fields. Married men and older people dominate farming or growing of crops in Kgatleng, Kweneng and Southern districts of Botswana and more females and youth should be encouraged to engage in farming activities. Most farmers fed harvested crop residues to domestic animals especially cattle and goats in forms ranging from *in-situ* grazing to hand-fed from the preparation of chopped residue mixed with other feed ingredients. This was carried out to improve their palatability and nutrition through the addition of ingredients such as protein sources like lablab and cowpeas residues, minerals like di-calcium phosphate and concentrates such as molasses. Addition of ingredients to crop residues was done after most farmers perceived maize residues to be of very poor quality while sorghum was rated better than other residues while the quality of millet was not known. Most farmers agreed with the idea of improving the quality of residues through bio-conversion process or mushrooms while some farmers only wanted to adopt mushroom production for generation of capital. Averaged across three replicate plots measuring 10m x 10m, estimated yields of maize, sorghum and millet crop residues were 12.13, 12.00 and 99.07 kg/ $100m^2$ respectively. Through provision of training and workshops farmers could know that mushroom production represents a valuable conversion of residues that can improve the productivity and performance of their livestock hence more profit can be made from selling both mushrooms and fattened livestock. Based on the above assertion; mushroom spent substrates (residues after cultivation of mushroom) were analysed for their nutritive value (Ramathele, 2017), diets formulated and analysed (Study 2) and tested for nutrient digestibility *in vivo* (Study 3).

3.7 Recommendations

Increasing the harvesting and processing of crop residues, mixing them with other feed ingredients is likely to meet feed requirements of grazing animals during the dry periods. Blending crop residues with high protein legumes such as cowpeas residues could reduce costs associated with supplementation intervention in Botswana as farmers will no longer buy costly protein supplements. This could improve the nutritive index of local crop residues and improve livestock productivity in the country.

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CHAPTER FOUR-STUDY TWO

4.0 The effect of crop residue type on the nutritional composition of oyster mushroom spent substrate-based diets for growing Tswana lambs

Abstract

Cereal stover contributes immensely to ruminant nutrition but are harvested when they are low in nutritive value, thus negatively affecting rumen fermentation and productivity. Corrective strategies involve ammoniation or legume supplementation. Low-cost oyster mushroom production for converting stover may increase its nutritive value and the sale of mushroom would add to farm income. The objective of this study was to determine the effect of crop residue type on the nutritional composition, *in vitro* and *in sacco* digestibility of oyster mushroom spent substrate based-diets for growing indigenous lambs. The study was arranged in a 3 x 2 factorial design with residue type ((millet stover (ML), sorghum stover (S) and maize stover; (MS)) as main plot (Factor A) and crop residue treatment (mushroom spent substrate and untreated) as a subplot (Factor B). All the treatment diets had the following basic ingredients (%): maize grain (19.0) soya meal (7.7 to 8.2), wheat bran (14.0), molasses (2.0 to 2.5), urea (0.7 to 1.9), di-calcium phosphate (0.2 to 0.3) and salt (0.2 to 0.3) and the six treatment diets were formulated by adding 55.0% of each of millet spent substrate based diet (MLSS), sorghum spent substrate based diet (SSS), maize stover spent substrate based diet (MSSS), millet stover based diet (ML), sorghum stover based diet (S) and maize stover based diet (MS). The nutritive values of the diets were determined using AOAC procedures. *In vitro* dry matter digestibility (IVDMD) of the diets was determined through incubation of test diets in Daisy^{II} incubator for 48 hours and *in sacco* degradability of dry matter (DM) was determined by incubating test diets in the rumen of two cannulated steers for 3, 6, 12, 24, 48, 72 and 96 hours. Cannulated steers were maintained on grass and lucerne ration (60:40 ratio) and given a period of 7 days to adapt to the diet before data collection. There were no significant differences in crude protein (CP) contents (15.56 and 15.42%) between mushroom treated and untreated substrate based-diets respectively, while mushroom treated substrate based diets had significantly lower ($P<0.05$) acid detergent fibre (ADF) (15.88% compared to 18.11% of the untreated diet) and acid detergent lignin (ADL) contents (7.16% compared to 8.65% of the untreated diet). There was an increase in IVDMD (from 79.72 to 85.57%) and organic matter digestibility (OMD) ($P<0.05$) (from 87.06 to 89.06%) due to the mushroom treatment effect. The MSSS diet had the highest OMD of 90.06% ($P<0.05$) compared to ML which had the lowest OMD of 84.68% among the diets. Significant interaction effect ($P<0.05$) was observed in CP with SSS having the highest content (15.92%) and lowest in other diets (15.09-15.67%). Fat (13.96%), percentage digestible energy (92.79%) and gross energy (21.96 MJ/Kg) were high ($P<0.05$) in SSS, MSSS and ML, diets respectively compared to other diets. Overall, mushroom treatment of cereal stover significantly increased ($P<0.05$) quickly degradable fraction (*a*), potential degradability (PD) and effective degradability ($ED_{0.03}$ and $ED_{0.05}$) of dry matter. The highest degradability response was for MSSS while ML had the lowest degradability parameters. The results indicate that bio-conversion of stover using oyster mushrooms increased fermentative characteristics and *in vitro* digestibility. This is likely to increase the supply of nutrients to the animal and improve productivity.

Keywords: crop residue type, mushroom spent substrate based-diets, nutritional composition, rumen fermentation dynamics, Tswana lambs

4.1 Introduction

In Botswana, ruminants largely survive on cereal crop residues to cope with feed scarcity during the dry periods experienced during winter months. However, the cereal straws are poor in available energy, protein and minerals that are required by grazing livestock (Abdou *et al.*, 2011). This is because most of the nutrients are locked in cell structures due to lignin complex which prevents utilisation of cell contents. Research devoted to how to improve the digestibility and utilisation of crop residues by ruminants has been conducted in developing countries. Efforts such as use of ammonia (Kim and Lee, 2005), hydrogen peroxide, wet oxidation (Chaturvedi and Verma, 2013), wood ash solution (Abdulazeez *et al*., 2016) and physical processing like grinding (Taylor and Bryant, 2007) have not been adopted by farmers either because they are expensive, inaccessible, have not been marketed and or are not environmentally friendly. A strategy of bio-conversion of crop residues through mushroom production has been a major focus of research (Yu *et al*., 2009; Akinyele *et al*., 2011; Mahesh and Mohini, 2013) in other countries and it provided interesting outcomes. Mushrooms or white rot fungi depolymerise stover as it is dependent on the digestibility of its structural carbohydrates (Fazaeli *et al*., 2002). Giovannozzi-Sermanii *et al*. (1989) also reported that degradation through the enzymatic mechanism of these molecules causes increased digestibility and availability of carbohydrates. Moreover, fungi in the genus *Pleurotus* possess ligninolytic activity which can improve digestibility and nutritional value of most straws and other crop residues (Kakkar *et al*., 1990; Kundu *et al*., 2005)). Due to the ligninolytic activity of oyster mushrooms (*Pleurotus* spp.), more protein with little cellulose and lignin, free sugars and more ash content are realised compared to the initial material (Kundu *et al*., 2005). Akinfemi *et al*. (2009) found that incubation of maize straw inoculated with two white rot fungi for 40 days converted straw into valuable ruminant feedstuff. The fungal filaments (mycelium) degraded the cellulose and hemicellulose thus making them more digestible by livestock (Akinfemi *et al*., 2009).

Conversely, Zadražil and Brunnert (1980) recorded *in vitro* dry matter digestibility (IVDMD) of up to 40 to 50 %in wheat straw treated with a strain of *P. ostreatus.* Calzada *et al*. (1987) recorded high dry matter digestibility when wheat straw was exposed to solid state fermentation by *P. ostreatus* for 30-days. Lignin content was significantly reduced and IVDMD increased from 14.3 to 29.5%. Ramirez-Bribiesca *et al*. (2010) treated corn straw with *P. ostreatus* for 15 days and results showed an increase in crude protein by 39.5% (3.44 to 4.80 g/100g), soluble protein by 165% (from 0.69 to 1.83 g/100g), soluble carbohydrates by 621% (from 0.37 to 2.77 g/100g), ash by 188.32% (2.74 to 7.90 g/100g) and decreased neutral detergent fibre by 14.5% (70.64 to 60.37 g/100g). In another experiment, a 50% increase in IVDMD was recorded after wheat straw was inoculated with *P. floridensis* (Sharma and Arora, 2010).

When peanut husk was treated with *Pleurotus ostreatus* and *P. pulmonarius,* CP content increased by 9.29% and 16.11% respectively, from 7.39% of the untreated peanut husk (Akinfemi, 2010). Moreover, fungal treatment of peanut husk depleted crude fibre. A similar trend, that is an increase in CP, was recorded by Akinfemi *et al*. (2009) after treating maize cobs and maize straw with white rot-fungi. In our laboratory, previous work by Ramathele (2017), showed an improvement in CP of maize stalk (from 4.38 to 7.4 %DM), decrease in ADL content in millet stalk (from 15.39 to 11.77 %DM) and decrease in NDF in sorghum (from 72.07 to 55.86 %DM) when the stover was treated with *Pleurotus* spp. These studies clearly indicate the nutritional improvement of poor quality straws by fungal treatment. However, it is not clear if diets formulated on spent mushroom stover or straw will have the same advantage. This is because formulated diets containing stover and a concentrate would display associative effects that may be positive or negative (Dixon and Stockdale, 1999). Negative associative effects occur when diets containing more concentrates divert digestion to produce more propionate (Bhatt *et al*., 2014) hence reducing fibre digestibility and production of short chain fatty acids which are needed for rumen motility and incorporation into milk protein (Madibela *et al*., 2005) respectively. Therefore, an appropriate balance between stover and concentrates is needed in formulated diets. The objective of the current study was to determine the effect of crop residue type on the nutritive value of Oyster mushroom spent substrate based-diets for growing lambs.

4.2 Materials and methods

4.2.1 Site description

The experiment was carried out at the Botswana University of Agriculture and Natural Resources (BUAN), Sebele, Gaborone. The University is located 24°36’ 40.90’’ S and 25° 56’ 13.35’’ E at 994m above sea level (Mojeremane *et al*., 2014). Laboratory work was done in the Biochemistry Laboratory in the Department of Animal Science and Production and mushroom production was carried out in low technology house in the BUAN Garden. The climate is semi-arid, with an average annual rainfall of 550 mm, and 6 to 7 months of dry period. In summer, temperatures vary from ~12–15 °C during the early morning, to ~30–40 °C by late afternoon in the hot, dry season (generally from mid-September to late October), but the maximum temperatures remain ~25–30 °C during the rainy season. The relative humidity is generally low, particularly in the dry months, (~0%), rising to an average of around 65%, in the rainy season as reported by Burgess (2006).

4.2.2 Collection of mushroom substrates

Dry maize, millet and sorghum crop residues were collected from BUAN farm and from farmers in Oodi and Malotwane identified during the farmer survey (Chapter 3). The residues were stored in a shade until ready for use.

4.2.3 Spawn preparation, substrate preparation and inoculation of substrates

Oyster mushroom mother spawns for *Pleurotus ostreatus*, *P. floridanus*, *P. ostreatus* x *P. floridanus*, *P. djamor* and *P. osteratus* strain HK35 were obtained from Professor E.B. Khonga, Department of Crop Science and Production, BUAN. To prepare spawn, sorghum grains were soaked for 24 hrs and boiled until they were soft and then water was drained. The grains were allowed to cool to room temperature and about 500 mL of the grain was transferred into heat-resistant 750 mL tomato sauce bottles. The grains were sterilized twice at 121°C in an autoclave (Front loader autoclave, TM 322-B85, Sturdy Industrial Co., Ltd) for 60 minutes and transferred into a clean laminar flow cabinet for inoculation with mother spawn. The laminar airflow cubicle was sterilized by spraying with 70% denatured alcohol before being wiped clean with a soft tissue. About 10-20 g of mother spawn was aseptically transferred into each bottle of sterile grain and the mouths of the bottles plugged with cotton wool. The spawn was allowed to run through the grain for 8 days at room temperature (24-28ºC) before being used for inoculation of mushroom substrates.

The crop residues were chopped using a chaff cutter into 2-4cm pieces. Each stover type was soaked separately in water for 24-36 hours. After draining excess water, the stovers were steamed in 200L metal drums in order to disinfect the substrate. After cooling, each steamed stover (~70% moisture content) was placed in polyethylene plastic bags (17 cm × 33 cm and 40µm thick) at a packing density of 2000 g of stover per bag and 5-10% (100-200g) of spawn was inoculated into each stover bag. Fifty culture bags were used for each stover type (to provide about 50 kg i.e. dry weight of the spent substrate for feeding trials). The inoculated substrates were kept in a mushroom house at 26-28°C and 60~70% relative humidity under a dark condition for 3-5 weeks for spawn running (Chun-Li *et al*., 2015). After spawn running, the bags were opened by cutting vertical slits into the sides of the plastic bags and bags were transferred to the growing house. The growing house was maintained at 22-24°C and 80-95% relative humidity. High humidity in the house was maintained by spraying water with a hosepipe on the floor and the walls of the house twice a day and an air conditioner was used to keep the temperature at 22-24°C. A total of three mushroom flushes were harvested before the spent substrates were used for diet formulations. At the end of the harvest period, for each residue or stover type, spent substrates of the various *Pleurotus* spp. were pooled together and sun-dried.

4.2.4 Preparation of spent mushroom substrates for chemical composition analysis

4.2.4.1 Experimental design

A 3 x 2 factorial completely randomised design (CRD) experiment, where residue type (Maize stover, Sorghum stover and Millet stover) was (Factor A) and residue treatment (mushroom spent substrate and plain stover) as Factor B was used in the study. A total of six diets (Treatments) were formulated based on previous work by Ramathele (2017) and three replicate samples per treatment were analysed for chemical composition as described in 4.2.5. The proportions of both stover and spent substrates were based on feedlot diets for beef cattle by Madibela *et al*. (2006). The experimental diets were formulated to support growth rates of 100 g/day.

Table 4.2.4.1. Dietary ingredients of experimental diets fed to growing Tswana lambs.

Feed ingredients (%)	Experimental diets [1]					
	SSS	MSSS	MLSS	MS	ML	S
Sorghum spent substrate	55.0	-	-	-	-	-
Maize spent substrate	-	55.0	-	-	-	-
Millet spent substrate	-	-	55.0	-	-	-
Maize stover	-	-	-	55.0	-	-
Millet stover	-	-	-	-	55.0	-
Sorghum stover	-	-	-	-	-	55.0
Maize grain	19.0	19.0	19.0	19.0	19.0	19.0
Soya meal	8.2	7.95	8.0	7.7	7.9	7.8
Wheat bran	14.0	14.0	14.0	14.0	14.0	14.0
Molasses	2.5	2.1	2.2	2.0	2.0	2.0
Urea	0.7	1.55	1.4	1.9	1.7	1.8
DCP	0.3	0.2	0.2	0.2	0.2	0.2
Salt	0.3	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100	100

[1] MLSS= millet spent substrate based diet, SSS= sorghum spent substrate based diet, MSSS= maize spent substrate based diet, ML= millet stover based diet, S= sorghum stover based diet, MS= maize stover based diet and DCP= di-calcium phosphate

4.2.5 Data collection

The following data expressed as percent (%) dry matter while energy parameters were in (MJ/kg) were collected:

1. Chemical compositions (dry matter, ash, organic matter, neutral detergent fibre, acid detergent fibre, acid detergent lignin, crude protein, fat, hemicellulose, non-structural carbohydrates, gross energy, digestible energy, metabolisable energy, tannins, organic matter digestibility and *in vitro* dry matter digestibility).

2. Mineral contents (Magnesium, Zinc, Copper, Iron, Potassium, Calcium, Sodium and Phosphorus)

3. Rumen fermentation dynamics (quickly degradable fraction of diet, the potentially degradable component of the diet, the rate of degradation, potential degradability and effective degradability at outflow rates of 0.03/h and 0.05/h).

Analysis of diets for chemical composition

Samples (600g) of each diet were weighed and then transferred to an air oven for drying at 60°C for 48 hours for dry matter (DM); method 930.15) analysis. After determination of DM, the samples were ground and passed through 2 mm sieve using a laboratory grinder (Fritsch Pulverisette 16, Idar-Oberstein, Germany). Samples in triplicates were analysed for Crude protein (CP) based on (AOAC 2005; method 954.01), ash (method 942.05, AOAC 2005) by combustion in the muffle furnace (Labcon Standard Furnace, muffle RM4, L23767) set at 600°C for 2 hours. Organic matter (OM) was calculated as 100 % - % Ash, non-structural carbohydrates calculated as 100 % – (%NDF+ %CP+ %fat+ %ash) (NRC, 2001), hemicellulose calculated as % NDF - % ADF (Jančík *et al*., 2008). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined sequentially according to the methods of Van Soest *et al*. (1991) using an ANKOM^{2000} Fibre Analyser (ANKOM Technology Corporation, NY, USA). During NDF determination, Alpha-amylase (Heat-stable bacterial alpha-amylase: activity =17,400 Liquefon Units / ml) (FAA, ANKOM Technology) and Sodium sulphite (Na_2SO_3), anhydrous (FSS, ANKOM Technology) were added. Acid detergent solution, 20g of cetyl-trimethylammonium bromide (CTAB) dissolved in 1L of 1.0 N H_2SO_4 was used during ADF determination. The acid detergent residue was treated with 72% sulphuric acid for lignin estimation.

Fat was determined using Ankom Technology (Ankom^{XT15} extractor), by boiling the samples in the die-ethyl ether for 60 minutes, to determine the weight loss of samples after fat extraction, then dividing it by initial sample weight multiplied by 100% to get %fat. Gross energy (GE) and digestible energy (DE) from samples incubated *in-situ* were determined using an adiabatic bomb calorimeter (Parr-328, Parr Instruments Co., Moline, IL, USA). The metabolisable energy was calculated as ME (MJ/kg DM) = 0.016 * DOMD, where DOMD indicates digestible organic matter per kilogram of dry matter (McDonald *et al*., 2011). Condensed tannins (proanthocyanidins) were determined according to a procedure by Porter *et al*. (1986), whereby a mixture of n-butanol, Hydrochloric acid (HCL) reagent and a 1.0-mL aliquot of the extract ferric ammonium sulphate in 2 mol/L HCl was added to extract (35 g) and contents vortexed. The extract was allowed to cool after boiling in a water bath for 60 minutes. The absorbance at 550 nm was recorded using UV-Vis spectrophotometer (Model UV-1601, Shimadzu Corporation, Japan).

To determine organic matter digestibility, the crucibles with the residue from *in vitro* incubated diets of treated and raw cereal stover were incinerated in the muffle furnace at 600°C for 2 hours and residual ash weighed the next day after cooling. Organic matter digestibility (OMD) was calculated after necessary corrections for blank samples. Organic matter digestibility was calculated as OMd = (feed OM− residue OM)/feed OM where feed OM and residue OM correspond to the organic matter measured in the feed and residue, respectively.

Animal handling, feeding, collection and processing of rumen liquor for *in vitro* dry matter digestibility

Animal care and ethics

Animals that donated rumen fluid and those in which nylon bags were incubated were cared for according to international guidelines for biomedical research involving animals (Council for International Organization of Medical Science-CIOMS, 1985).

Two rumen cannulated steers kept at the Department of Agricultural Research kraals were maintained on a grass and lucerne (60%: 40%) diet. They were allowed to adapt to the diet for

7 days and were offered feed once daily, in the morning (0900 hours). Rumen fluid samples for *in vitro* dry matter digestibility (IVTDMD) were collected before feeding in the morning at 0800 hours and combined. Two 2-litre thermos bottles were pre-heated by filling them with warm (39°C) water. Heated water was emptied just prior to collection of rumen inoculum. At least 2000 mL of rumen inoculum was strained through three layers of cheesecloth and mixed in a pre-warmed CO_2-filled thermos after collection at the laboratory to maintain an anaerobic environment for rumen microbes' survival. The rumen inoculum from the thermos was emptied into a blender. The blender container was purged with a CO_2 gas and rumen inoculum blended at a high speed for 30 seconds. The blending action served to dislodge microbes that are attached to the mat and assure a representative microbial population for the *in vitro* fermentation.

Buffer solution consisted of two solutions prepared as described by ANKOM Technology (2005), (solution A and solution B; Ankom Technology Corporation, Fairport, NY, USA). Solution A was made of 20g of KH_2PO_4, 1.0g $MgSO_4.7H_2$, 1.0g NaCl, 0.2g $CaCl_2.H_2O$ and 1.0g Urea (reagent grade) in 2 Litres of distilled water while buffer Solution B consisted of 15g Na_2CO_3 and 1g $Na_2S.9H_2O$ in 1 litre of distilled water. Both buffer solutions (A and B) were pre-warmed to 39^{o}C by placing them in a water bath set at 39^{o}C before use. In separate containers, 266 mL of solution B was added to 1330 mL of solution A (1:5 ratio). The exact amount of solution A to B was adjusted to obtain a final pH of 6.8 at 39°C. Four hundred millilitres (400 ml) of rumen inoculum in a graduated cylinder was added to the mixture of solution A and B (1:4 ratio).

The temperature of daisy digestion jars was allowed to equilibrate to 39°C for at least twenty to thirty minutes. F57 filter bags (ANKOM, Technology, with a pore size of 2µm) were pre-rinsed in acetone for three to five minutes and completely air-dried in open lab. The acetone rinse was to remove a surfactant that may inhibit microbial digestion. About 0.25g of each of the formulated diet was weighed in triplicate into fibre bags, sealed using Impulse bag sealer-ANKOM Technology - 1915/1920 heat Sealer and placed in the Daisy^{II} Incubator digestion jar (up to 25 samples per jar). Samples were evenly distributed on both sides of the digestion jar divider with two blank bags also being incubated and used as a correction factor. *In vitro* dry matter digestibility was then determined by incubating the diet samples for 48 hours at a temperature of 39.5°C ± 0.5 in ANKOM Technology - DAISY^{II} Incubator. At the end of 48 hours, the jars were removed and fluid drained. Bags were thoroughly rinsed with cold tap water until the water was clear. Rinsed bags were put into the ANKOM200/220 Fibre analyser (Ankom Technology Corporation, Fairport, NY, USA) and Van Soest *et al*. (1991) procedure for determining NDF was followed.

In vitro dry matter digestibility (IVDMD)

IVTDMD was calculated as the difference between DM incubated and the residue after NDF analysis, using the following formula;

% IVTDMD= $(100 - (W3 - ((W1 \times C1))/W2 \times 100$

Where W_{1}= Bag tare weight (g), W_{2} = Bag + sample weight (g),

W_{3} = Final bag and fibre weight (g) after NDF extraction

C_{1}= Blank bag correction = final oven dried weight / original blank bag weight

Analysis for minerals

About (1.25g) of each diet sample was digested using sulphuric acid–selenium (Se) digestion mixture following the procedure by Sahrawat *et al*. (2002) to determine Calcium (Ca), Phosphorus (P), Magnesium (Mg), Zinc (Zn), Iron (Fe), Copper (Cu), Sodium (Na) and Potassium (K) levels. Inductively coupled plasma optical emission spectrometer (ICP-OES) (Model Optima TM 2100 DV, Perkin Elmer ® precisely, Germany) was used to read Ca, Mg, Zn, Fe, Cu, Na and K, while P was read using UV-Vis spectrophotometer (Model UV-1601, Shimadzu Corporation, Japan) and absorbance, was determined at 670nm wavelength following Molybdenum blue method of Dickman and Bray (1940).

In situ rumen degradation and rumen fermentation dynamics

In situ rumen degradation of diets based on mushroom spent substrates and original straw was evaluated in two rumen cannulated steers – (Tswana breed) (~450± 50 kg BW). The steers were housed in an open pen, with diets and water under separate shades and diets contained grass and lucerne at a ratio of 60%: 40%. The steers were allowed to adapt to the diets for a period of 7 days before collection of data started. The dried and ground (through 3 mm screen mesh) diet samples (3 g of sample) were weighed into three nylon bags (6.5 x 14 cm with a pore size of 41 µm; Bar Diamond Inc. USA) per time point and incubated in the rumen of steers for different periods, i.e., 0, 3, 6, 12, 24, 48, 72 and 96 hours. During removal, bags were dipped in cold water and placed in a bucket containing ice water and later placed in a freezer until all bags had been removed. This was done to avoid further degradation of feed particles by rumen microbes. Zero hours' samples were not incubated but washed with others to estimate water soluble-material. Thereafter the bags were washed in cold water under a running tap till the rinse solution becomes clear. This was done to prevent further fermentation and wash off the feed particles adhering to the outside of the bags. The nylon bags were then dried at 60°C for 48 hours as described by Karsli and Russell (2002). The bags were cooled in a desiccator for 10 minutes. The rumen degradation of DM was calculated by subtracting the residue sample weight from the original sample weight incubated. The data was initially plotted against time to determine if there was a lag time. Upon observation of lack of lag time, the following equation was used.

In situ or time series degradation data were fitted into Ørskov and McDonald (1979) equation;

$P = a + b\,(1 - e^{-ct})$

Where;

P = the DM degradation after time 't'.

a = quickly degradable/soluble fraction of diet at time zero.

b = the potentially degradable component of the diet that with time will be degraded.

c = the rate of degradation of 'b'.

The total degradation of diets is given by **a + b** which obviously cannot exceed 100. It follows that **100 - (a+b)** represents the fraction which will appear to be un-degradable in the rumen/bag (Ørskov *et al*., 1980).

Effective degradability (ED) was estimated according to the equation of **ED = a + bc / (c+*k*)** (Ørskov, 1992), where ***k*** is the fractional outflow rate from the rumen, which was assumed to be 0.03/h or 0.05/h. Rumen outflow rate of 0.03/h and 0.05/h is that which is expected to occur in maintenance and productive ruminants respectively but not necessarily lactating dairy cows or triplets bearing/nursing ewes where outflow rate of 0.08/h would be used. Degradation constants (*a*, *b*, *c*, PD, $ED_{0.03}$ and $ED_{0.05}$) of DM were estimated by general non-linear models (NLIN) procedures (SAS, 2002-2008) using a SAS program written by the Rowett Research Institute (Osuji *et al*., 1993).

4.2.6 Data analysis

Data on chemical composition, mineral contents and rumen degradation parameters were subjected to analysis of variance (ANOVA) using general linear models (GLM) procedures of SAS (2002-2008) and if the f-values were significant at $P \leq 0.05$, means were separated using the least squares means separation (Ott, 1967) which was performed using the PDIFF option of GLM Procedure in SAS (2002-2008) to evaluate the significance and magnitude of the fixed effects at $P \leq 0.05$. All data were expressed on dry matter basis and expressed as means ± standard error, with the experiment being duplicated and the analysis made in two runs.

The following statistical model was applied:

$$Y_{ij} = \mu + F_i + T_j + (F_i * T_j) + \varepsilon_{ij}$$

In which;

$\boldsymbol{Y_{ij}}$ = response variable

$\boldsymbol{\mu}$ = population mean

F_i = forage type effect; i = (sorghum, maize and millet forages)

T_j = treatment method effect; j = (mushroom treated or colonised and untreated)

$(T_i * F_j)$ = interaction effect (forage type X treatment method)

ε_{ij} = random error $N(0, \sigma^2)$ (population mean, variance)

4.3 Results

4.3.1 The effect of forage type and treatment method on nutritional composition of lamb's diets

Averaged across the stover treatment, there were no significant differences in CP (15.36-15.66%), ash (4.90 -5.38%), OM (94.62-95.10%), Tannin (0.13-0.15%) and IVDMD (81.53-84.85%) contents among sorghum, maize and millet stovers while there were significant differences ($P \leq 0.05$) observed for the other parameters. The contents of the fibre fraction of the cell wall (NDF, ADF, and ADL) varied among forages. Neutral detergent fibre content of maize (54.37%) was significantly lower than those of sorghum (60.71%) and millet (61.69%) which were similar while ADF content was significantly lower in sorghum forage (15.96%) than in millet while that of maize (17.17%) was not significantly different from the other two. Millet stover had significantly higher ADL content of 9.81% than those of maize (7.10%) and sorghum (6.81%) which were similar. Forage colonised by mushroom mycelium was significantly lower than the untreated, respectively in DM (92.11 and 96.22%), ADF (15.88% and18.11%), ADL (7.16% and 8.65%), and tannins (0.11% and 0.16%) contents. Treating forage with mushroom mycelium had no effect ($P>0.05$) on ash, organic matter, crude protein and gross energy of the forages.

Table 4.3.1. The main effects of forage type and treatment method on the chemical composition of formulated diets (%DM) while GE, DE and ME are expressed in (MJ/kg).

	Parameter evaluated (%DM)														
Effect [2]	**DM**	**ASH**	**OM**	**NDF**	**ADF**	**ADL**	**OMD**	**CP**	**FAT**	**HEM**	**GE**	**DE**	**ME**	**TAN**	**IVDMD**
Forage [3]															
S	92.97^{b}	5.27	94.73	60.71^{a}	15.96^{b}	6.81^{b}	89.34^{a}	15.66	11.36^{a}	44.75^{a}	18.66^{b}	16.09^{b}	5.98^{a}	0.15	81.53
MS	95.10^{a}	4.90	95.10	54.37^{b}	17.17^{ab}	7.10^{b}	88.77^{a}	15.43	9.78^{c}	37.20^{b}	19.36^{b}	17.14^{a}	5.94^{a}	0.13	84.85
ML	94.43^{a}	5.38	94.62	61.69^{a}	17.86^{a}	9.81^{a}	86.06^{b}	15.36	10.30^{b}	43.83^{a}	21.13^{a}	17.04^{a}	5.76^{b}	0.13	81.56
SEM	0.189	0.211	0.211	0.330	0.419	0.224	0.289	0.136	0.066	0.617	0.220	0.230	0.019	0.010	1.012
P value	<.0001	0.2655	0.2655	<.0001	0.0159	<.0001	<.0001	0.2955	<.0001	<.0001	<.0001	0.0079	<.0001	0.271	0.0496
Treatment method [4]															
Mushroom	92.11	49.77	95.02	60.12	15.88	7.16	89.06	15.56	11.79	44.23	19.60	17.20	5.96	0.11	85.57
untreated (control)	96.22	53.88	94.61	57.73	18.11	8.65	87.06	15.42	9.17	39.62	19.84	16.31	5.83	0.16	79.72
SEM	0.154	0.172	0.172	0.269	0.342	0.183	0.236	0.111	0.054	0.504	0.180	0.190	0.015	0.008	0.827
P value	<.0001	0.1087	0.1087	<.0001	0.0002	<.0001	<.0001	0.3785	<.0001	<.0001	0.3684	0.0036	<.0001	0.0002	<.0001

^{abc} Means in the same column, with the same superscripts, are not significantly different at ($P \geq 0.05$).
Where**; SEM**= standard error of mean

[1]**DM**= Dry matter, **OM**= Organic matter, **NDF**= Neutral detergent fibre, **ADF**= Acid detergent fibre, **ADL**= Acid detergent lignin, **OMD**= Organic matter digestibility, **CP**= Crude protein, **HEM**= Hemicellulose, **GE**= Gross energy, **DE**= Digestible energy, **ME**= Metabolisable energy **TAN**= Tannins, **IVDMD**= *In vitro* dry matter digestibility

[2] **Effect**; two main effects were investigated and they were forage type and treatment method.

[3] **S**= Sorghum stover based diet, **MS**= Maize stover based diet, **ML**= Millet stover based diet.

[4] **Treatment method**; the stover was either treated through mushroom (spent substrate) or used as it was (control).

Across interactions between stover types and treatment methods, there were no significant differences in ash (4.83-5.89%), organic matter (94.11-95.35%), ADF (14.52-18.52%), ADL (6.16-10.59%), DE (15.27-17.47 MJ/Kg), tannins (0.09-0.18%) and IVDMD (79.07-88.87%) contents among colonised and untreated millet, maize and sorghum stovers, while there were significant differences ($P \leq 0.05$) observed for the other parameters.

Table 4.3.2. The interaction effect of forage type and treatment method on the chemical composition of six formulated diets based on stover and spent mushroom substrates expressed on (%DM basis) while GE, DE and ME are expressed in (MJ/Kg).

Effects [4]		**Parameter evaluated (%DM)**														
Forage [2]	**Treatment method [3]**	**DM**	**Ash**	**OM**	**NDF**	**ADF**	**ADL**	**OMD**	**CP**	**FAT**	**HEM**	**GE**	**DE**	**ME**	**TAN**	**IVDMD**
ML	mushroom	92.72[c]	4.88	95.12	61.79[a]	17.30	9.04	87.44[b]	15.09[b]	11.47[b]	44.48[a]	20.30[b]	17.20	5.85[b]	0.09	83.87
	none	96.14[a]	5.89	94.11	61.59[a]	18.42	10.59	84.68[c]	15.64[b]	9.12[d]	43.17[a]	21.96[a]	16.87	5.67[c]	0.17	79.25
MS	mushroom	94.06[b]	4.83	95.17	58.39[b]	15.83	6.29	90.06[a]	15.67[b]	9.94[c]	42.56[a]	18.83[cd]	17.47	6.03[a]	0.11	88.87
	none	96.12[a]	4.97	95.35	50.34[c]	18.51	7.91	87.49[b]	15.20[b]	9.62[c]	31.84[b]	19.89[bc]	16.80	5.86[b]	0.14	80.84
S	mushroom	89.55[d]	5.22	94.78	60.17[ab]	14.52	6.16	89.68[a]	15.92[a]	13.96[a]	45.66[a]	19.66[bc]	16.91	6.00[a]	0.12	83.98
	none	96.39[a]	5.31	94.69	61.24[a]	17.41	7.46	89.01[ab]	15.40[b]	8.76[d]	43.84[a]	17.66[d]	15.27	5.95[a]	0.18	79.07
SEM		0.268	0.298	0.298	0.468	0.593	0.317	0.409	0.192	0.094	0.873	0.032	0.032	0.027	0.001	1.431
CV(%)		0.57	11.51	0.63	1.59	6.98	8.01	0.93	2.49	1.79	4.16	3.21	3.85	2.25	21.95	3.46
P-value		<.0001	0.2499	0.2499	<.0001	0.2884	0.8739	0.0360	0.0193	<.0001	<.0001	<.0001	0.1379	0.0360	0.2235	0.4333

[abcde] Means in the same column, with the same superscripts are not significantly different at ($P \geq 0.05$).

Where**; SEM**= standard error of mean
CV (%); Coefficient of variation
[1] **DM**= Dry matter, **OM**= Organic matter, **NDF**= Neutral detergent fibre, **ADF**= Acid detergent fibre, **ADL**= Acid detergent lignin, **OMD**= Organic matter digestibility, **CP**= Crude protein, **HEM**= Hemicellulose, **GE**= Gross energy, **DE**= Digestible energy, Metabolisable energy, **TAN**= Tannins, **IVDMD**= *In vitro* dry matter digestibility
[2] **S**= Sorghum stover based diet, **MS**= Maize stover based diet, **ML**= Millet stover based diet
[3] **Treatment method**; the stover was either treated through mushroom (spent substrate) or used as it was (control).
[4] **Effect**; two main effects were investigated and they were forage type and treatment method.

The content of magnesium in sorghum (2.49 mg/kg) and maize (2.42 mg/kg) forage was similar but higher than that of millet (2.18 mg/kg) while zinc content of sorghum (85.23 mg/kg) and millet (80.34 mg/kg) was similar but higher than maize (52.59 mg/kg) forage. The content of copper was 6.09 mg/kg in sorghum forage and 5.93 mg/kg in millet, with both forages being significantly higher than maize forage (4.95 mg/kg). Millet forage had the highest amounts of iron (1.62 mg/kg) followed by sorghum (0.32 mg/kg) and maize (0.23 mg/kg) which were similar but different from millet forage.

Forage colonised by mushroom mycelium was lower than the untreated ones respectively, in contents of magnesium, iron and potassium. However, zinc (78.68 and 66.76 mg/kg), Ca (3.66 and 3.02 g/kg), sodium (4.07 and 1.36 g/kg) and phosphorus (1.24 and 0.70 g/kg) contents were significantly higher in treated forage than the untreated forage respectively. Mushroom treated and untreated forage had the same ($P>0.05$) amounts of copper.

Table 4.3.3. The main effect of forage type and treatment method on mineral content of lambs diets based on sorghum, maize and millet stover.

Effect [2]	**Minerals** [1]							
	Mg (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (g/kg)	K (g/kg)	Na (g/kg)	P (g/kg)
Forage [3]								
S	2.49^{a}	85.23^{a}	6.09^{a}	0.32^{b}	3.32^{b}	12.24^{b}	4.27^{a}	1.35^{a}
MS	2.42^{a}	52.59^{b}	4.95^{b}	0.23^{b}	3.70^{a}	13.31^{a}	1.90^{b}	1.23^{b}
ML	2.18^{b}	80.34^{a}	5.93^{a}	1.62^{a}	3.00^{c}	13.42^{a}	1.97^{b}	0.33^{c}
SEM	0.05	1.93	0.26	0.139	0.08	0.32	0.05	0.04
P value	0.0023	<.0001	0.0111	<.0001	<.0001	0.0339	<.0001	<.0001
Treatment method [4]								
Mushroom	2.17	78.68	5.80	0.40	3.66	10.86	4.07	1.24
Untreated (control)	2.56	66.76	5.50	1.04	3.02	15.12	1.36	0.70
SEM	0.04	1.58	0.20	0.11	0.07	0.26	0.04	0.03
P value	<.0001	<.0001	0.3222	0.0009	<.0001	<.0001	<.0001	<.0001

[abc] Means in the same column, with the same superscripts are not significantly different at ($P \geq 0.05$).
Where**; SEM**= standard error of mean
[1] **Mg**= Magnesium, **Zn**= Zinc, **Cu**= Copper, **Fe**= Iron, **Ca**= Calcium, **K**= Potassium, **Na**=Sodium, **P**=Phosphorus
[2] **Effect**; two main effects were investigated and they were forage type and treatment method.
[3] **S**= Sorghum stover based diet, **MS**= Maize stover based diet, **ML**= Millet stover based diet.
[4] **Treatment method**; the stover was either treated through mushroom (spent substrate) or used as it was (control).

In general, mushroom treated forages had the highest mineral contents compared to untreated forages. Untreated maize-based forage diet had the highest (2.91 mg/kg) content of magnesium, followed by colonised sorghum (2.51 mg/kg). Contents of calcium and phosphorus were high in colonised sorghum stover based diet compared to other diets.

Table 4.3.4. The interaction effects of forage type and treatment method on the mineral content of six formulated diets based on plain stover and spent mushroom substrates.

Forage [2]	Treatment method [3]	Minerals [1]							
		Mg (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (g/kg)	K (g/kg)	Na (g/kg)	P (g/kg)
ML	Mushroom	2.09^{cd}	72.53^{b}	5.69^{ab}	0.40^{b}	3.51^{b}	10.44^{bc}	2.62^{b}	0.34^{e}
	None	2.28^{bc}	88.15^{a}	6.16^{ab}	2.85^{a}	2.49^{c}	16.40^{a}	1.32^{de}	0.32^{e}
MS	Mushroom	1.92^{d}	64.17^{b}	4.81^{b}	0.34^{b}	3.33^{b}	9.75^{c}	2.22^{c}	1.42^{b}
	None	2.91^{a}	41.00^{c}	5.08^{b}	0.12^{b}	4.08^{a}	16.87^{a}	1.59^{d}	1.04^{c}
S	Mushroom	2.51^{b}	99.36^{a}	6.91^{a}	0.48^{b}	4.15^{a}	12.37^{b}	7.37^{a}	1.96^{a}
	None	2.48^{b}	71.11^{b}	5.27^{b}	0.16^{b}	2.50^{c}	12.10^{b}	1.18^{e}	0.74^{d}
SEM		0.076	2.74	0.36	0.197	0.11	0.455	0.07	0.05
P value		<.0001	<.0001	0.0163	<.0001	<.0001	<.0001	<.0001	<.0001

[abcde] Means in the same column, with the same superscripts are not significantly different at ($P \geq 0.05$).
Where**;** **SEM**= standard error of mean
[1] **Mg**= Magnesium, **Zn**= Zinc, **Cu**= Copper, **Fe**= Iron, **Ca**= Calcium, **K**= Potassium, **Na**=Sodium, **P**=Phosphorus
[2] **S**= Sorghum stover based diet, **MS**= Maize stover based diet, **ML**= Millet stover based diet.
[3] **Treatment method**; the stover was either treated through mushroom (spent substrate) or used as it was (control).

Forage type did not have any effect ($P>0.05$) on a quickly degradable fraction (*a*), insoluble but potential degradable fraction (*b*), rate of degradation (*c*) and potential degradability **(**PD) constants of dry matter. However, $ED_{0.03}$ was high in maize (47.07 h^{-1}) and sorghum (42.85 h^{-1}) forage than in millet (39.80 h^{-1}) forage while $ED_{0.05}$ was also high and similar in maize (39.89 h^{-1}) and sorghum (36.02 h^{-1}) forage than in millet (33.00 h^{-1}) forage. Quickly degradable fraction (*a*) (22.85 and 13.77%), PD (83.84 and 74.26%), $ED_{0.03}$ (46.73 and 39.75 $h^{-1)}$ and $ED_{0.05}$ (39.88 and 32.74 h^{-1}) of the dry matter were higher in treated forage than the untreated forage respectively. (*b*) and *c* constants were not affected ($P>0.05$) by treatment method.

Table 4.3.5. The main effects of forage type and treatment method on rumen fermentative dynamics of complete diets based on sorghum, millet and maize stover, either treated (spent substrate) or untreated (control).

	Degradability constants [1]				ED at different passage rates [2]	
Effect [3]	*a* (%)	*b* (%)	*c* (%/hr)	PD	*k*=0.03	*k*=0.05
Forage [4]						
ML	16.34	61.04	0.019	77.39	39.80[b]	33.00[b]
MS	19.86	60.57	0.025	80.43	47.07[a]	39.89[a]
S	18.73	60.61	0.020	79.33	42.85[a]	36.02[a]
SEM	1.19	0.34	0.003	1.34	1.40	1.18
P value	0.1844	0.5817	0.4521	0.3374	0.0288	0.0177
Treatment method [5]						
Mushroom	22.85	60.98	0.019	83.84	46.73	39.88
Untreated (control)	13.77	60.50	0.023	74.26	39.75	32.74
SEM	0.97	0.28	0.003	1.09	1.14	0.97
P value	0.0006	0.2684	0.3704	0.0008	0.0050	0.0020

[ab]Means in the same column, with the same superscripts, are not significantly different at ($P \geq 0.05$).
Where: **PD**= Potential degradability and **SEM**= standard error of mean
[1] Constants ***a***, ***b*** and ***c*** are described by the equation $P= a+ b\ (1- e^{-ct})$, where P is the dry matter disappearance at time t; a is the zero-time intercept; $a + b$ = the total degradability; c is the rate of degradation (h^{-1}).
[2] **ED** is the effective degradability calculated using the equation $ED= a + (bc / (c+ k))$, where k is the outflow rate from the rumen assumed to be either 0.03 or 0.05 h^{-1}.
[3] **Effect**; two main effects were investigated and they were forage type and treatment method.
[4] **S**= Sorghum stover based diet, **MS**= Maize stover based diet, **ML**= Millet stover based diet.
[5] **Treatment method**; the stover was either treated through mushroom (spent substrate) or used as it was (control).

Insoluble but a potential degradable fraction (*b*) and (*c*) were not affected by an interaction between forage type and treatment method. However, colonised maize-based forage diet had higher (26.13%) *a* degradability constant than other diets.

Table 4.3.6. The interaction effect of forage type and treatment method on the rumen fermentative dynamics of complete diets based on sorghum, millet and maize stover, either treated (spent substrate) or not treated (control).

Forage [3]	Treatment method [4]	Degradability constants [1]			PD (%)	ED (%) at different passage rates [2]	
		a (%)	*b* (%)	*c* (%/hr)		k=0.03	k=0.05
ML	Mushroom	20.25^{c}	61.25	0.02	81.49^{c}	42.70^{c}	36.03^{c}
	None	12.45^{f}	60.84	0.02	73.29^{f}	36.91^{f}	29.97^{f}
MS	Mushroom	26.13^{a}	61.01	0.02	87.14^{a}	51.67^{a}	44.56^{a}
	None	13.59^{e}	60.14	0.03	73.72^{e}	42.47^{d}	35.24^{d}
S	Mushroom	22.20^{b}	60.69	0.02	82.88^{b}	45.83^{b}	39.04^{b}
	None	15.26^{d}	60.53	0.02	75.78^{d}	39.87^{e}	33.01^{e}
SEM		1.682	0.482	0.005	1.90	1.98	1.675
P value		0.0066	0.6762	0.6921	0.0100	0.0197	0.0090

[abcdef] Means in the same column, with the same superscripts, are not significantly different at ($P \geq 0.05$).
Where: **PD**= Potential degradability and **SEM**; standard error of mean
[1] Constants ***a***, ***b*** and ***c*** are described by the equation $P= a+ b\ (1- e^{-ct})$, where P is the dry matter disappearance at time t; a is the zero-time intercept; $a + b$ = the total degradability; c is the rate of degradation (h^{-1}).
[2] **ED** is the effective degradability calculated using the equation $ED= a + (bc / (c+ k))$, where k is the outflow rate from the rumen assumed to be either 0.03 or 0.05 h^{-1}.
[3] **S**= Sorghum stover based diet, **MS**= Maize stover based diet, **ML**= Millet stover based diet.
[4] **Treatment method**; the stover was either treated through mushroom (spent substrate) or used as it was (control).

4.4 Discussion

4.4.1 Effect of forage type and treatment method on the chemical composition of complete diets based on cereal stover

Forage type effect

The difference in DM levels, even when the diets had similar ingredient composition could be due to the dense stem pith of maize straw hence more bagasse which elevates the DM levels. The differences could also be due to the difference in days to maturity and harvesting between plant species. In a study by Babu *et al*. (2014), complete diets based on sorghum and maize stover had DM contents of 93.00 and 93.80 %DM respectively. The results of that study and the present study are comparable.

Ramana Reddy *et al*. (2016) recorded NDF content of 77.93 %DM in maize stover complete diet while Babu *et al*. (2014) recorded NDF content of 76.60, 75.20 and 76.48 %DM in Sorghum stover, Maize stover and Sweet sorghum-based complete rations for Nellore lambs. Neutral detergent fibre contents of experimental complete diets in the current study were not comparable to those reported by the authors as they were low. Variation in NDF content of complete diets in the studies could be due to different sampling times that would even influence NDF of samples from the same variety or even within the same plant (Hanson and Rivera, 2010). Neutral detergent fibre constitutes insoluble fibres, cellulose and lignocellulose which reflects the total bulk of the feedstuff and can be used as an indicator of intake potential (Nepomuceno *et al*., 2018). These are carbohydrate sources which are metabolised through microbial fermentation in the reticulo-rumen to liberate volatile fatty acids and used as energy source by the animal (Castillo-Gonzáleza *et al*., 2014).

Venkateswarlu *et al*. (2014) reported ADF and ADL levels of 29.51 and 5.24 %DM respectively in complete diets based on sorghum straw included at 50% rate. The ADL content of the present study (Table 4.3.1) are close to the ones observed by Venkateswarlu *et al*. (2014), however, there is a difference in ADF contents of complete diets in both studies. Maize stover complete ration had ADF levels of 42.54 %DM (Babu *et al*., 2014) and ADL levels of 6.51 %DM (Ramana Reddy *et al*., 2016). Differences in ADF content could be attributed to the higher content of roughage used for the formulation of complete rations. McDonald *et al*. (2011) emphasised that the recommended ADF in feeds is 19% DM and levels below this level may fail to arouse the saliva secretion and reduce the buffering capacity of the rumen liquor. This finding was supported by Krause *et al*. (2002), who found out that chewing activity and saliva production are only triggered by fibre fraction of the diet in dairy cows. Therefore, the diets used in the present study may not elicit sufficient chewing and hence enough saliva production to adequately buffer the rumen environment.

Gebrehawariat *et al*. (2010) observed OMD levels of 63.00 %DM in maize stover based diet which was lower than of the present study. Njidda and Nasiru, (2010) stated that differences in OMD are due to variations in CP and NDF concentrations among the diets. This was inconsistent with the findings of the present study as no variations (P>0.05) in CP content of diets was observed but only in NDF content. This perhaps suggests that in the present study NDF had the highest effects. High OMD in S and MS can be an indication of enhanced nutrients availability which might support the turnover rate of rumen microbes. Conversely, low OM digestibility of ML forage-based diet may be connected to anti-nutritional factors**.** Kumar *et al*. (2016) stated that finger millet contains tannins and protease inhibitors, which might directly or indirectly inhibit degradability of nutrients in millet or millet based diets**.** However, tannin levels were the same in all forages. The presence of these anti-nutritive factors could affect the bioavailability of nutrients, therefore, making it impossible for microbes to utilize ML forage-based diet for the production of useful volatile fatty acids, the major source of energy to the ruminant livestock, (Getachew *et al*., 1998).

In a study by Venkateswarlu *et al*. (2014), hemicellulose content in complete diets containing different amounts of sorghum straw ranged from 24.71 to 28.25 %DM. Those findings by Venkateswarlu *et al*. (2014) disagree with that of the present study (Table 4.3.1). High hemicellulose levels recorded in the present study could be due to the high concentration of respective nutrients in the roughage and type of concentrate used for the formulation of complete rations. With feed ingredients such as yellow maize and wheat bran having been added during formulation of complete diets, hemicellulose levels increased, leading to more of it being hydrolysed together with cellulose and other cell constituents. According to McDonald *et al*. (2011), most of the glucose that is utilised as an energy source is from cellulose and hemicellulose with the latter providing galactose and xylose which are metabolised to yield energy.

The results show that all the diets had high energy levels and most of the energy was digestible (Table 4.3.1). In the present study, ML had the highest gross energy compared to the other forages. This lower *in situ* degradability of ML corroborate the low *in vitro* dry matter digestibility and organic matter digestibility discussed above. Other anti-nutrients beside high fibre components that would undermine degradability of energy could be tannins and other secondary metabolites which were not tested in the present study. It is a widely documented that condensed tannins interfere with microbial fermentation in the rumen (Naumann *et al*., 2017) hence the expectation that if ML had tannins then the same effect would be observed. However, S diet had the lowest energy content but most of it was digestible when compared to that of other diets suggesting that microbial function was not interfered with. This could be due to the amount of lignin which protects the cell contents from being hydrolysed to produce energy, which was lower in S forage-based diet. Lowest energy degradability which was recorded in ML was accompanied by the highest ADL content, suggesting a negative association. Improved degradability of feeds is desirable hence it is essential to know the digestible energy that can be metabolised by an animal. The results of the present study are different from that of Babu *et al*. (2014) who found ME levels of 8.84±0.63 and 8.88±0.54 MJ/kg in complete diets based on sorghum and maize stover respectively. The variation between the studies could be due to the degree of digestibility which is influenced by the proportion of lignin and soluble carbohydrates in a feed. It can be concluded that any feed can have high gross energy but not most of it can be metabolised and be available for use by the animal.

Fat findings of the present study differ with of Babu *et al*. (2014) who reported EE levels of 1.26 and 1.31 %DM in sorghum and maize stover complete diets. Even though in the latter study the oiled rice bran, groundnut cake and sunflower cake were included during diet formulation, fat levels were significantly lower than that of the present study. This could be due to the low inclusion rate of sources of fat in the diets per kilogram. In the present study, the main source of fat which was soybean meal was included at a rate of 7.7, 7.8 and 7.9 %/kg in maize, sorghum and millet forage based complete diets respectively compared to 0.0238 %/kg included in both sorghum and maize stover based diets in study by Babu *et al*. (2014). This resulted in dilution and variation in fat levels reported. High-fat content increases the energy density of the diet which is needed by rumen microbes for efficient utilisation of the feeds (Marn *et al*., 2013). However, high fat intake can reduce dry matter intake and fibre digestion which in dairy cows' is triggered by gut peptides release in response to extra fat (Marn *et al*., 2013). Reduced feed intake may be caused by increased rumination time associated with negative effects on microbial degradability of the feeds (Chilliard *et al*., 1993).

Forage effect was not significant on crude protein, Ash, Organic matter, tannins and IVDMD. Information on chemical attributes of complete rations based on millet stover was scarce and its chemical composition did not vary much from complete diets based on maize and sorghum stover. That is why it was not discussed more or compared to the findings of other authors. Experiments or studies on using millet straw in complete diets could be conducted to generate data since the chemical properties or attributes of millet straw are known.

Treatment method effects

There was a loss of DM which was a 4.27% decrease due to treatment effect (Table 4.3.1). Reductions in DM content could be linked to the extent at which the forage was soaked in water before being used in mushroom production or the level at which the fungi degraded the cell contents hence reducing biomass of the forage source in the diets. Dry matter levels ranging from 75.0 to 75.9 %DM were recorded in total mixed rations containing spent wheat straw compared to 75.5 %DM recorded in a complete diet containing untreated forage Shahzad *et al*. (2016). During mushroom farming dry matter losses depend mostly on the strain of mushroom used, the length of mushroom production and the type of substrate used (Tuyen *et al*., 2013).
Omidi-Mirzaee *et al*. (2017) reported a reduction in NDF content from 71.3 to 54.7 %DM in a complete diet where rice straw was a source of forage while in wheat straw based diet it reduced from 81.7 to 56.7 %DM. The reported results contradict that of the present study as an increase in NDF was observed. However, the NDF fraction of the diet in the reported study by Omidi-Mirzaee *et al*. (2017) was more extensively degraded, with NDF variations being associated with amounts of water-soluble carbohydrates in different residues that were used as mushroom substrates. Seok *et al*. (2016) recorded OMD levels of 53.6 %DM in by-product feed based roughage diet compared to 55.37 %DM recorded in the rye straw based diet which was not treated. With high OMD reported in the present study, the results suggest that treating stover

for use in compounding complete diets has the likely effects of reducing the ruminating time and improving feed intake.

There was a reduction in ADF and ADL content due to treatment effect in the present study. Similar findings were reported by Fazaeli *et al*. (2004) after including fungal treated wheat straw in the diet of the late lactating cow where ADF levels reduced from 35.60 to 33.80 %DM while ADL reduced from 7.40 to 6.30 %DM. Acid detergent lignin levels were comparable to that of the present study, while the difference was observed in ADF content between the present study and that of Fazaeli *et al*. (2004). Differences in ADF reduction of the experimental diets could be due to strain of mushroom or the length at which the forage used in diets formulations were exposed to fungal colonisation. The forage used in the present study was colonised by fungi for maximum of 35 days compared to 50 days taken in the study by Fazaeli *et al*. (2004). Reduction in ADF and ADL contents of the diets indicates that fungi improved nutritive values of the diets by lowering cell wall contents. This is because of fungi from *Pleurotus* spp. which possess ligninolytic activities which can depolymerise lignin complex structures hence reductions in fibre levels (Kundu *et al*., 2005).

Treatment increased hemicellulose content (Table 4.3.1). In contrast hemicellulose levels decreased from 18.3 to 12.6 %DM in by-product feed-based silage diet in a study by Seok *et al*. (2016). The findings of Seok *et al*. (2016) disagree with the present one as an increase in hemicellulose was observed. With high starch content in forages leading to increased efficiency of cellulose hydrolysis, it might have led to high cellulose conversion ratio which is defined as starch yield + cellobiose yield (Solange *et al*., 2008). Conversely, reduction in hemicellulose is due to secretion of lignocellulosic enzymes such as laccase, xylanase, cellulase (Phan and Sabaratnam, 2012). These lignocellulosic enzymes have efficient hydrolysis mechanism which targets hemicellulose in forages due to the spatial proximity of synergistically acting enzymes hence being incorporated in foreign enzymatic activities to enhance further hydrolysis of lignocellulose in the production of chemicals and plant-derived biofuels (Davidi *et al*., 2016). It is important in animal nutrition that any microorganism used to upgrade the quality of forage-based diets must have the ability to degrade lignin, with low degradation of hemicellulose (FAO, 2011). McDonald *et al*. (2011) highlighted that hemicellulose is linked to cellulose (cell wall polysaccharides) and are utilised well by rumen microflora as an energy source for the synthesis of protein. Therefore, an increase in hemicellulose in this study was welcome observation.

As forage was treated, digestible energy (DE) of complete diets increased. Babayemi *et al*. (2006) reported high yields of short chain fatty acids from mushroom treated straw used in diets hence an improvement in the energy content of the diets. According to Keller *et al*. (2003), the breakdown of lignin-carbohydrate linkage by fungi releases carbohydrates which are fermented to release energy in the rumen. This implies that a vast energy potential of the diets is unlocked in cereal crop residues and treatment like mushroom cultivation on the crop residues will unlock such energy. The higher DE of the treated forage-based diet might also be explained by reduced ADF and ADL content in the diet in comparison to the untreated forage-based diet. There are a few data in the literature regarding analysis for *in situ* digestible energy in complete diets based on spent cereal stover substrates. This could be one of the parameters which researchers can include in addition to proximate analysis of the diets as it can be used to estimate the amount of the energy that can be metabolised by an animal in the rumen.

In comparison with the untreated diet, the inclusion of the fungal treated forage increased fat or ether extract content in diets (Table 4.3.1). These results are supported by Fazaeli *et al*. (2004) who reported fat levels of 1.70 and 1.80 %DM in untreated and treated wheat straw based diet respectively. Moreover, fat levels of 3.9 %DM were reported by Seok *et al*. (2016) in by-product feed-based silage, which was composed of 45 % spent mushroom substrate compared to 0.2 %DM in rye straw based diet. However, the results of the compared studies are different. When comparing the fat content of the diets in the present study, they were higher than that of the reported studies by Fazaeli *et al*. (2004) and Seok *et al*. (2016). This variation could be due to different ingredients and their proportions in formulated diets. In the present study, soybean meal was the main source of fat and included at a rate of 7.7 to 8.0% in diets while protected fat was included at 1.0 % rate in a study by Seok *et al*. (2016). Park *et al*. (2012) observed a decrease in the fat content of complete diets when inclusion of spent mushroom substrates in diets was increased. The decrease in fat content could be due to the dilution factor brought about by increased inclusion rate of SMS. Therefore, manipulating diets' containing SMS to have improved fat levels which will not affect feed intake by animals (<6%) will be essential in improving feed utilisation by microbes. This can be achieved through reduction of SMS inclusion in diets to prevent dilution factor or including protected fats.

The *in vitro* dry matter digestibility of mushroom treated forage-based diet was 85.57 %DM and of the untreated-forage based diet was 79.72 %DM (Table 4.3.1). The *in vitro* digestibility of DM in the present study was increased by 6% units which were comparable to that found (10%) by Fazaeli *et al*. (2002) in cattle feeding on fungal treated wheat straw based diets. Therefore, *P. ostreatus* have the potential to upgrade the nutritive worth of forage-based diets. With increased IVDMD, it might be an indication of the cell wall (ADF and ADL) break down due to white rot fungi (Zadražil and Puniya, 1995). Improved IVDMD due to lignin degradation was also experienced by Shahzad *et al*. (2016), who reported an increase in dry matter digestibility of complete rations from 64.5 to 69.3 %DM. It can be stated that treated cereal stover has improved dry matter digestibility (DMD) which could lead to an improved turnover rate of rumen microbes when fed to livestock, hence high intake and digestibility of diets. Conclusion made from the results of the present study is that, lignocellulosic enzymes which are mostly secreted by *Pleurotus ostreatus* during mycelium growth and mushroom production such as α -Amylase; EC 3.2.1.1 and Cellulase; 3.2.1.4, (Ko *et al*., 2005) significantly degrade cell wall components thus exposing contents to be utilised by rumen microbes.

From Table 4.3.1 no differences were seen in Ash, OM, CP and GE due to treatment effect. However, in many studies Akinfemi *et al*. (2009a); Akinfemi *et al*. (2009b); Akinfemi (2010) CP content of spent crop residues based diets improved. Increase in crude protein levels is due to increased fungal biomass (Chen *et al*., 1995) which might not have occurred in the present study.

4.4.2 Forage type and treatment method interaction effect on chemical composition of complete diets based on cereal stover

All diets containing untreated forage i.e. ML, MS and S had the highest DM content compared to the diets with treated forage (Table 4.3.2). Therefore, treatment of forage before its inclusion in diets reduced DM levels of the complete diets. This was due to forage being subjected to water hydration during sterilisation. The findings of the current study are in accordance with of Park *et al*. (2012) who reported DM losses in diets containing spent mushroom substrates (SMS). In a study by Park *et al*. (2012) dry matter levels ranged from 71.96 to 73.14 %DM when SMS was included at 15-20 % rate in diets compared to dry matter content of 74.94 of the control diet which did not contain any SMS. A similar trend of decrease in DM content was also observed by Park *et al*. (2012a) after inclusion of varying levels of SMS in complete diets for Elk. Reductions in DM content of SMS containing diets could be due to heat generated (Buckmaster and Dennis, 2010) during microbial degradation of bagasse and lignin causing a decrease in structural carbohydrates and cell wall components.

All mushroom treated generally had elevated contents of NDF compared to diets in which forage was not treated (Table 4.3.2). Park *et al*. (2012a) observed no difference in NDF content of diets, regardless of whether they contained SMS as a forage source or not. However, in a study by Seok *et al*. (2016), NDF levels reduced from 46.7 to 39.9 %DM due to the inclusion of SMS. In the present study, NDF content of all diets was higher than of the reported studies. Neutral Detergent Fibre levels should not be more than 48.0-50.0 %DM as they decrease feed consumption rates (Van Soest, 1982) and all experimental diets had NDF content above this recommended levels. However, high NDF levels could be beneficial as they promote rumen health through improved rumen turnover rate, which is required to stimulate saliva production (Harper and McNeil, 2015) to buffer the rumen. Moreover, the high polysaccharides content of the diets which are degraded by rumen fauna such as protozoa, bacteria and fungi will be important in subtropical grazing systems where NDF requirements are above 40%.

Significant interaction effects were not pronounced between MLSS, ML, MSSS, SSS and S. However, MS differed greatly in hemicellulose from the other diets (Table 4.3.2). Harikrishna *et al*. (2012) reported hemicellulose levels ranging from 19.32 to 19.92 %DM in complete diets with sorghum straw at 50% inclusion rate and varied levels of thermotolerant yeast. Since hemicellulose is a component of NDF, it varies greatly across plant species and within a plant as it matures. This could be the reason for differences in hemicellulose content of the two compared studies. Sources of forage in the present study might have been harvested at early stages of maturity than in the other study hence high hemicellulose content. Moreover, ingredients used in diets formulations such as maize grains had a great influence on hemicellulose levels as they are highly soluble. Hemicellulose is completely digested in the rumen when not protected by lignin and in the present study, this could be a possibility as lignin was reduced in diets containing treated forage. The results mean the treated cereal stover based diets could yield nutrients which will be available to the animals.

The differences between the CP content of the diets are not much pronounced even though they are reported to be significant (Table 4.3.2), with the highest CP being of SSS diet. The diets were formulated to be iso-nitrogenous and were intended to supply 15.0% crude protein. The discrepancy in CP amounts of the diets could be brought by underestimation of the protein content of ingredients used to formulate the diets, especially soybean meal. This is because soybean meal was not analysed for CP content, but literature was used to get its protein content. This could have led to the underestimation of its protein hence more supply of protein which did not differ much across all diets. All diets were able to meet protein requirements of growing lambs. Even though there is a tendency of SSS having the highest CP content, its nitrogen was utilised the same as of other diets used in study 3. Moreover, it did not influence the weight changes of the lambs in study 3, since they were the same across all lambs fed the experimental diets.

In a study by Shahzad *et al*. (2016), there were no differences in crude protein levels of complete diets based on wheat straw. Reported CP levels were 13.6, 13.7 and 13.6 %DM in total mixed rations containing different levels of fungal treated wheat straw compared to 13.7 %DM. When sorghum was included in complete rations, (Venkateswarlu *et al*.,2014), reported CP levels were 10.51 %DM compared to 15.40 %DM reported in the present study. Variations are connected to the type of dietary true protein which was included in the diets. In the current study, soybean meal was used as a protein source while in the other study groundnut cake was used to supplement the diets and they had different amounts of protein. Sharifi Hosseini *et al*. (2015) reported CP increment from 11.0 to 11.3 %DM in complete diets based on untreated and treated wheat straw respectively. During fungal treatment, there is a capture of excess nitrogen (Sallam *et al*.,2007) which increases CP content of treated diets. Therefore, treated forage based complete diets could be a good source of protein for livestock.

Treatment of forages increased the fat contents of all diets with the highest increase being observed in sorghum forage (8.76 to 13.96 %). This was also reported by Fazaeli *et al*. (2004) who stated that inclusion of fungal treated straw up to 30% of the total mixed ration increased fat contents of the rations which were also recognised by an increase in the fat amount of milk by 13% in late lactating Holstein. Increased fat levels led to the improved digestibility of nutrients and average daily weight gains 2.7 times (Fazaeli *et al*., 2004). Oh *et al*. (2010) investigated the effects of dietary SMS supplementation such as *Pleurotus eryngiia* in Hanwoo steers and the findings were that fat amounts of the TMR containing SMS had increased fat content (2.19%) compared to 1.28% of the TMR based on rice straw. Increased fat levels in complete diets containing SMS could be due to the presence of enzymes with lipolytic activity during mycelium colonisation of substrates causing hydrolysis of triacylglycerol to glycerol and constituent fatty acids (Chavan and Kadam, 1989). According to Chavan and Kadam, (1989), the increased lipase activity results in improvements in the contents of total proteins, fat, certain essential amino acids, total sugars, B-group vitamins, and a decrease in anti-nutrients. The findings from the present study imply that when spent mushroom substrates from cereal stover are included in complete diets there seemed to improve fat levels of the diets. This means SMS would be able to be utilised as low-quality roughage and could effectively be used as microbial additives for beef cattle (Kim *et al*., 2012).

A complete diet based on sorghum straw had OMD level of 90.87 %DM (Harikrishna *et al*., 2012) and the OMD value was almost similar to the one recorded in the present study (Table 4.3.2). However, the OMD findings recorded by Venkateswarlu *et al*. (2014) were inconsistent with the findings of the present study. The OM digestibility of complete diet based on sorghum was 61.03%DM compared to 69.8 %DM in by-product based silage containing 45% SMS. Increased digestibility of OM in diets could be due to the proportion of the concentrates in the diets which is positively correlated to OM (Cantalapiedra-Hijar *et al*., 2009). With an improvement in OMD of treated diets, they have the potential to provide the minerals which are required by ruminants since most of the mineral matter is digested. There are a few data in the literature regarding the degradability of energy in complete diets.

Sorghum straw based diet had a gross energy content of 8.78 MJ kg^{-1} DM in a study by Harikrishna *et al*. (2012). Due to the destruction of carbohydrates and lignin complex structures during substrates colonisation, soluble carbohydrates levels increased hence increase in energy content of the sorghum forage-based diets (Table 4.3.2). There was a reduction in energy levels of treated diets in the present study. The reduction could be brought about by some fungi utilising some of the soluble carbohydrates hence reduction in sources of energy. Moreover, more than 76.00% of the gross energy was digestible (Table 4.3.2). The metabolisable energy was 10.04 MJ kg^{-1} DM in wheat straw based diet compared to 10.50 MJ kg^{-1} DM in treated wheat straw based diet (Shahzad *et al*.,2016). Different energy sources were used in the compared studies, hence variations in energy yields which led to them being metabolised differently. Since most of the energy from treated diets is metabolised, it will be used for productive purposes.

The interaction effect of forage type and treatment method was not significant (P>0.05; Table 4.3.2) for Ash, OM, ADF, ADL, DE, Tannins and IVDMD. However, in most cases, there was an insignificant increase in IVDMD of spent residues based diets after biological treatment of crop residues with *Pleurotus* spp. This is attributed to better or improved ligninolytic activity of the mushroom. The findings of the present study indicate that there was a negative though an insignificant relationship between IVDMD and ADF fraction of the formulated diets. Digestible energy also increases when crop residues are treated through mushroom since the rumen microbes are able to attach to cell contents as cell wall structures are degraded. The conclusion reached from the present study was that the nutritive composition of experimental diets varied greatly with the treatment method. The highest response due to treatment method was seen in MS forage-based diet.

4.4.3. The main effect of forage type, treatment method and forage type by treatment method interaction on the mineral content of complete diets.

Forage type effect

The main effect of forage type and treatment method on the mineral content of formulated diets based on sorghum, maize and millet stover are shown in Table 4.3.3.

The reported Ca and P contents of complete diets are in line with of Kumar (2014) as values in range as 2.58 and 2.46 g/kg were reported for Ca and P respectively. However, they were low when compared to the ones reported by Boonsaen *et al*. (2017), with differences being attributed to the inclusion of mineral premix which could have increased mineral contents in the latter study. Legodimo (2013) observed Ca content of 0.053 and 0.049 g/kg in sorghum and millet-based complete diets respectively, with diets formulated to provide 15.5 % crude protein. Phosphorus contents were 0.033 and 0.030 g/kg. Generally, calcium, Zn and Cu contents of the forage-based diets were within the recommended critical range of minerals for grazing animals McDowell (1992) while P, K and Fe were not. Macro minerals such as calcium are associated with phosphorous metabolism in animals (Madibela and Modiakgotla, 2004), Zn being component of enzymes and Cu is needed in blood compounds as it is present in certain plasma proteins, such as ceruloplasmin which helps with the release of iron from the cells into the plasma (McDonald *et al*., 2011). The evaluated forage-based diets will be a potential source of these minerals.

Treatment method effect

An increase in minerals content of treated forage was supported by Medina *et al*. (2009); Zhu *et al*. (2012) who stated that spent substrates are rich in minerals which are vital to animal health such as Fe, Ca, Zn and Mg. Most of the animals' metabolic processes occur because of minerals and they should be provided in the right amounts. Fazaeli *et al*. (2004) reported Ca levels of 0.065; 0.069 % and P levels of 0.046; 0.048 % in untreated wheat straw; fungal treated wheat straw based complete diets. The reported results of this study are not in line with of the current study (Table 4.3.3). The treated forage-based diets in the present study provide fifty-five times more than the amount of Ca and P recorded in tested diets by Fazaeli *et al*. (2004). Spent substrates have high inorganic matter which contributes to the high mineral matter of the treated forage-based diets. The high inorganic matter is a result of some minerals such as P not leaching out during the weathering process caused by microbial activity (Beyer, 1999). The significance of availability of Ca and P from the treated forage-based diets will be felt in the reproduction of grazing ruminants and local farmers as supplementations in forms of mineral licks (Madibela *et al*., 2002) will be reduced. These two minerals constitute the bulk of mineral supplementation in Botswana in the form of Di-calcium Phosphate licks (APRU, 1980). Soils in Botswana are low in P, hence it is also low in the natural pasture as well (Madibela *et al*., 2000).

Forage type by treatment method interaction effects

Sorghum spent substrate based-diet had the highest Ca and P content compared to the other diets while ML had the lowest Ca and P levels (Table 4.3.4.). In general, the SSS diet had the highest levels of all minerals and can meet the mineral requirements of most livestock. This will help in providing a conducive environment for the functioning of the many enzymes and coenzymes of the various metabolic pathways. The ratio of Ca to P in all diets was more than 2:1 ratio recommended for the physiological function of phosphorous metabolism of bones (McDowell,1992; Abdulrazak *et al*., 2000). It was also found out that all the diets can meet calcium requirements of lactating animals, with Copper levels of all diets in the present study being within the range of 4.0-8.0 mg/kg for temperate pasture grasses (McDonald *et al*., 2011). Meeting Cu requirement will prevent high cases of copper deficiency syndromes in grazing ruminants as Cu is a component of blood protein like erythrocuprein which plays a significant role in oxygen metabolism (McDonald *et al*., 2011).

4.4.4. The main effect of forage type and treatment method and interaction effect of forage type by treatment method on rumen fermentative dynamics of complete diets based on cereal stover

Forage type effect

The results for the quickly soluble fraction (*a*), potentially degradable fraction of forage (*b*), rate of degradation of (*c*), Potential degradability (PD) of forage are presented, with fractional outflow rates 0.03 and 0.05 % per hour presented below the Effective degradability (ED) of dry matter (DM) (Table 4.3.5). The effective degradability estimated with ruminal passage rates of 0.03 and 0.05 h^{-1} were consistently higher for MS diet and lower for ML. Therefore, maize stover based diet was better in terms of DM degradability followed by S diet. In a study by Raja Kishore (2012), maize stover complete diet had ED_5 level of 35.27 % while other complete rations based on stover ranged from 34.97 to 35.40 % and were comparable to the ones reported in the present study. High ED_3 and ED_5 recorded in maize stover based diet could be due to stem pith which occupies more area within the stem segment, hence less silica and lignin which inhibits microbial degradability of cell contents. Feeding maize stover based diet means the retention time of the diet will be reduced hence more feed intake. In general, increasing ruminal outflow rate from 3 to 5% reduced the ED but did not change the trends or patterns observed in the different forage-based diets.

Treatment method effect

All forage-based diets showed improved *a*, PD and ED_3 and ED_5 degradability constants (Table 4.3.5). The potentially degradable component of diets that with time will be degraded (*b*) and the rate of degradation (*c*) were the same across the tested forage, meaning that treatment did not have any effect on them. Overall, compared to the untreated forage-based diets, higher *a*, PD and ED_3 and ED_5 of the treated forage-based diets explain the effectiveness of *Pleurotus* spp. treatment in reducing the lignin content of forages included in diets. Increase or improvement in degradability constants was expected because of changes in the ratio of cell walls to cell contents, caused by the fungi. Mushroom treated forages have high cell soluble content hence better degradability by the rumen microbes. Montañez-Valdez *et al*. (2015) recorded an increment in (*a*) fraction (from 35.7 to 48.9 %) and (*c*) (from 0.023 to 0.027 %), a decrease in PD (from 73.6 to 72.9 %) as maize stover in diets was treated with *Pleurotus djamor*. The reported results disagree with of the present study since there was an increase in PD and reported (*a*) in the present study is lower than that of the author. Factors such as fungal strain, length of mushroom cultivation and conditions in mushroom cultivation normally affect or have an influence on the extent in which substrates are degraded by fungi (Montañez-Valdez *et al*., 2015).

Forage type by treatment method interaction effects

The potentially degradable component of diets (*b*) and the rate of degradation (*c*) were similar (P>0.05) for all tested complete diets (Table 4.3.6). The quickly degradable fraction of the diet (*a*) was high in MSSS compared to other diets, with ML diet having the lowest (*a*) fraction. The (*a*) fraction is too high for stover based diets because crop residues are devoid of NSC and therefore "*a*" does not exist. This could be an indication of errors in measurement or the contribution of maize grains and molasses in the diets. Moreover, adding SMS could not change the value significantly (Table 4.3.6). Raja Kishore (2012) observed (*a*); 10.13%, PD; 62.16 % and ED_5; 35.27 % in maize stover complete diets, with ED_5 being the same with of the current study. However, *a* and PD kinetics were lower than of the present study. The possible reason to support the difference in results could be different stages of harvest and season which turn to affect the quality of the residues (Van *et al*., 2002). These differences lead to inhibition of microbial activity and prevent forages or diets from being digested to their full potential. The PD of forage is normally influenced by protein supplement and its linkage with the rumen ecosystem (Ruiz *et al*., 2009), with high protein content being positively correlated to high degradability constants. In overall, bio-conversion of cereal crop residues increased soluble fraction and effective degradability at the two outflow rates with the greatest response for ED parameters being for maize stover.

From the previous results of study 2 (Chapter four; Table 4.3.2, 4.3.5 and Table 4.3.6) the highest response in terms of value addition or bio-conversion of cereal stover, was observed in maize stover and the lowest was seen in millet stover. Minerals were high in Sorghum stover.

Hence a decision was taken to use MSSS and SSS as diets to be fed to growing Tswana lambs in digestibility study. Non-spent complete diets based on S and ML stover were not used in the Study 3 experiments (Chapter five) because their nutrient digestibility was low compared to the three spent stover based diets. However, MS was used as a control diet because more of the stover that was collected from respondents in the first study (chapter 3) was maize. Moreover, maize crop was the most cultivated crop during the time the study was conducted hence MS was chosen as a control in Study 3 (Chapter 5).

4.5 Conclusions

Employing biological treatment of crop residues improves their feeding value. However, inclusion of SMS in complete diets would require taking into account factors such as nutrition level of the SMS, digestibility and voluntary intake. The degradation of the complex carbohydrates by the fungus increases the level of soluble carbohydrates which result in increase in organic matter digestibility, digestible energy and metabolizable energy in the fermented products. The calcium and phosphorus contents of the *Pleurotus* spp. fermented substrates based diets are also significantly enhanced. Therefore, the use of SMS as feed ingredient has several merits as it is a local resource that could reduce feeding costs during in countries that import hays like Botswana. Farmers can greatly benefit from feeding SMS as fodder since it is more digestible than the raw crop residues from arable farming. Moreover, the problem of accumulation of crop residues during winter could be solved.

Important variations of *in sacco* degradability parameters were reported among treatments. Treatment through mushroom or bio-conversion of cereal stover reduced fibre components but increased fermentative characteristics and *in vitro* digestibility of crop residues. This was due to degradation through the enzymatic mechanism which made carbohydrates content available and reduced lignin hence improving digestibility of the residues and supply of nutrients to the rumen. Bio-conversion increased soluble fraction and effective degradability at the two outflow rates, with maize having the highest degradability parameters compared to other forages which could improve the growth performance of livestock.

4.6 Recommendations

Feeding spent maize and sorghum-based complete diets to indigenous lambs are likely to increase the supply of nutrients to the animal and improve productivity. This is because bio-conversion of stover reduced fibre components and increased fermentative characteristics and *in vitro* digestibility. Therefore, small-scale farmers should employ biological treatment to improve nutrient utilisation of stover by rumen the ruminant animals, which is abundant after periods of harvesting. Moreover, importation of good sources of roughages for use in complete diets will be reduced.

4.7 References

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CHAPTER FIVE-STUDY THREE

5.0 The effect of mushroom spent substrates-based diets from local cereal crop residues on *in vivo* nutrient digestibility, nitrogen balance and growth of Tswana lambs

Abstract

Feeding mushroom spent substrate based-diets to ruminants could be a practice that would provide a source of improved roughage for livestock in Botswana and improve productivity. However, such feedstuff should be tested for nutrient digestion, nitrogen intake and utilisation on the desired class of animals. This will inform strategies on how to use them and also inclusion levels that would meet nutrient requirements for growth. The study was conducted to evaluate the nutrient digestibility, nitrogen utilisation and growth performance of yearling Tswana lambs fed mushroom spent substrate based-diets. Twelve (12) castrate Tswana lambs aged between 9-12-months were first blocked by initial body weight into three groups and the groups randomly assigned to three dietary treatments with maize stover based diet being the control (MS), n = 4; weight; 17.1kg), maize spent substrate based-diet (MSSS), n = 4; weight; 18.8kg) and sorghum spent substrate based-diet, (SSS), n = 4; weight; 17.3kg). The lambs were fed individually in pens and later transferred to the metal metabolic crate, arranged in a completely randomised design (CRD) with four lambs per treatment. Each lamb was offered 3% body weight which was approximately 600g/ day and provided with clean water *ad* libitum for a period of 42 days with the last 12 days used for nutrient digestibility study (5 days for adjustment to the metabolic crates and 7 days for data collection). There were no differences ($P>0.05$) in final body weights of lambs fed the three diets. However, there were significant increases in total body weight over time in animals fed all the diets. No significant differences ($P>0.05$) were observed at any weekly weighing point throughout the experiment. There were no significant differences ($P>0.05$) in total weight gain (TWG), average daily gain (ADG) and feed conversion ratio (FCR) observed. Lambs fed SSS diet had the highest daily feed intake

(DFI) ($P<0.05$) of 586.00 g/day which was not different from lambs fed MSSS (581.50 g/day) but higher than of lambs fed MS which had DFI of 464.70 g/day. Significant differences ($P<0.05$) were observed in nutrient digestibility of Dry matter (DM); MS, MSSS and SSS (55.84%, 51.72% and 48.12% respectively), digestibility of acid detergent fibre (ADF) was higher in lambs fed MS (51.82 %) and lowest in lambs that consumed MSSS (44.50%) while acid detergent lignin (ADL) digestibility was highest ($P<0.05$) in lambs offered MSSS (29.46%) and SSS (26.36%) diet and lowest in those fed MS (19.33%). Digestibility coefficients of fat (87.89%) and calcium (84.00%) were significantly higher ($P<0.05$) in lambs fed SSS diet compared to other diets. Phosphorus digestibility was high (95.00%) in lambs offered MS, followed by MSSS (91.57%) and lowest in lambs on SSS (88.38%). Nitrogen intake amounts of 14.38 and 14.39 g/day were observed in lambs fed MSSS and SSS respectively which were significantly higher ($P<0.05$) than of lambs fed MS diet (11.15 g/day). A similar pattern was observed in nitrogen balance and retention. The conclusion reached was that inclusion of mushroom spent substrates in complete diets did not support better growth performance or final body weights of Tswana lambs than the control. However, it improved feed intake and digestibility of some nutrients by lambs. The limiting factor for high growth and differences may have been due to high inclusion rate (55%) of the roughage for the growth of Tswana sheep, therefore future studies may test low inclusion rate of spent mushroom substrates in complete diets.

Keywords: growth performance, mushroom spent substrate based diets, nitrogen balance, nutrient digestibility, Tswana lambs

5.1 Introduction

In most developing countries like Botswana, the inclusion of agricultural by-products to animal diets during the dry periods has increased. This could be linked to increased feed costs which accounts for 70% (Ajayi *et al.*, 2007) of production costs and also the need to tackle environmental issues (Park *et al.*, 2012) posed by agricultural waste. Mushroom spent substrates from mushroom production are one of the common agricultural by-products which have been used in livestock ration formulations elsewhere (Silvana *et al.*, 2006; Kim *et al.*, 2011) but not in Botswana. They are alternative sources of protein when included in rations, with a potential of providing 16.11% crude protein in fungal treated peanut husk compared to 7.39 % of the untreated husk (Akinfemi, 2010). Hence they can be mixed with other feed ingredients to supply 15% crude protein which is required by lambs for growth (Rabelo *et al.*, 2003). With modernised protein evaluation systems, the actual requirement and supply could be determined (Gao *et al.*, 2015). Moreover, it is necessary to be sure if the nutrients in feeds can fulfil the requirements of an animal and this can be proven by conducting digestibility trials. The trials are done to prevent over or undersupply of nutrients required by animals. It has been demonstrated in some cases where increased supply of crude protein (CP) did not improve the production and performance of dairy cattle and this was caused by more supply of rumen degradable protein (RDP) and highly soluble carbohydrates (Harstad and Prestløkken, 2001). Oversupply of a nutrient such as nitrogen results in contamination of the environment when these are excreted in urine and/or manure (Bashir *et al.*, 2013). Digestion trials are therefore conducted to assess the nutrient requirements of animals to facilitate the formulation of their rations. To effectively carry out the trials, it is essential to know the total requirements of the animal's tissues for each nutrient and the capacity of the diet to provide the tissues with these required nutrients (Pereira *et al.*, 2012). In a study by Cherney *et al*. (2004), feeding diets containing more than 50% of dry matter (DM) as high-quality forage to highly productive dairy cows were shown to support nutrient requirements for milk production. This was because high-quality forages have high digestibility and can be consumed in greater quantities than low-quality forages that have high NDF and low digestibility (Llamas-lamas and Combs, 1991). Pereira *et al*. (2009) stated that favourable strategies like appropriate feeding management and use of intensive systems can help in improving animal production during the dry season. But for resource-limited small-scale farmers, conventional intensive systems are not practical and viable. Therefore, the use of nutritional and low-cost feeds like mushroom spent substrates (as has been proven by Ramathele, 2017 and Chapter 4 above) that utilise crop-livestock farming system may be the way of the future. This is essential as mushroom spent substrates have increased CP and degraded cell wall components (Fazaeli and Masoodi, 2006) compared to the original straw and this could be beneficial to ruminants. Spent wheat straw from *Agaricus bisporos* included in diets up to 25% increased nutrients digestibility but reduced dry matter intake (DMI) in diets of buffalos. The nutrients requirements are assumed to correspond with the protein intake on which the response is measured using standards such as live weight or nitrogen retention and done by measuring the nitrogen (N g/day) consumed and excreted in the faeces, and in the urine (Safwat *et al*., 2015). The Department of Crop Science and Production of Botswana University of Agriculture and Natural Resources has pioneered low-technology mushroom production system using local cereal crop residues with *Pleurotus* spp. but there is a lack of integration with livestock feeding management. However, before using such feedstuff there is a need to test them for nutrient digestion, nitrogen intake and utilisation on the desired class or species of livestock. Therefore, this study was conducted to evaluate the growth performance, nutrient digestibility and nitrogen utilisation of growing Tswana lambs fed mushroom spent substrate based-diets

5.2 Materials and methods

5.2.1 Site description

The experiment was carried out at the Botswana University of Agriculture and Natural Resources (BUAN) small stock kraals near Estate Management Unit (EMU) Sebele, Gaborone, from September to November 2017. The site is 24°35'04.7"S, 25°56'36.1"E at an altitude of 991 m (Legodimo, 2013). The climate is semi-arid, with an average annual rainfall of 550 mm, and 6 to 7 months of dry period; in summer, temperatures vary from ~12–15 °C during the early morning, to ~30–40 °C by late afternoon in the hot, dry season (generally from mid-September to late October), but the maximum temperatures remain ~25–30 °C during the rainy season, low humidity, particularly in the dry months, (~0%), rising to an average of around 65%, in the rainy season as reported by Burgess (2006).

5.2.2 Animals, diets and experimental design

A total of twelve (12), 9-12-months-old, castrated indigenous growing lambs sourced from Botswana University of Agriculture and Natural Resources (BUAN), Notwane farm were blocked by body weight into three groups made of four lambs per treatment as replicates. The groups were randomly allocated to three treatment diets; maize stover based diet (MS) weighing 17.1±1.46kg, maize spent substrate based-diet (MSSS) weighing 18.8±1.46kg and sorghum spent substrate based-diet (SSS) weighing 17.3±1.46kg. The lambs were fed individually in covered pens (1.5m x 1m), arranged in a completely randomised design (CRD). The pens were open sided, well-ventilated, with concrete floor thoroughly washed and cleaned using disinfectant (Savlon) and allowed to dry, then equipped with feed and water troughs. The lambs were vaccinated against pasturella and pulpy kidney diseases (Pasturella-2ml/lamb and pulpy kidney -1ml/lamb small stock vaccines, Onderstepoort Biological Products company, South Africa). The lambs were also dewormed with albendazole 1.9% (Valbazen ® Pfizer, South Africa). Ecto-parasites were checked after 7 days and Ivermectin 1% (Ecomectin ®, Intervet, South Africa) used when there was an infection. Each lamb was offered a weighed amount of feed at the rate of 3% of its body weight daily and fresh water provided all the time. The diets contained 15% crude protein that met the protein requirement of growing indigenous lambs weighing up to 10 kg live weight (National Research Council, 2007). The feeding trial lasted for 42 days after an acclimatization period of five (5) days. The ingredients used and the chemical composition of all experimental diets including the control which is the untreated maize stover forage based diet are shown in Chapter 4 (Table 4.2.4.1 and 4.3.2) respectively.

5.2.3 Performance indices

The lambs were weighed at the beginning of the experiment and subsequently on weekly basis to evaluate average weight changes before feeding in the morning. The weights (feed and lambs) were measured using CFW 150 electronic platform weigh scale (±20 kg, Adam Equipment 2006 – Software version V1.04). Data on feed intake and weight gain was then determined. Feed intake was obtained by subtracting left-over feeds from the quantity offered (Yashim *et al*., 2016) each week to obtain weekly feed intake per treatment.

The following formulae were used to calculate other performance indices;

Total weight gains (TWG) = Final weight (kg) - initial weight (kg).

Average daily gain (ADG) = initial and final live weight differences (TWG) in (g) ÷ Total number of experimental days.

Feed conversion ratio (FCR) = Daily feed intake (DFI) ÷ ADG (Malik *et al*., 1996).

5.2.4 Assessment of nutrient digestibility and nitrogen balance

At the end of the growth study, all the growing lambs were weighed and transferred to individual metabolic crates with facilities for separate collection of faeces and urine. The metabolic crates were equipped with a drinker and a manual feeder. Experimental diets fed to the animals were the same as those used in the growth study. An adjustment period of 5 days was allowed to acclimatise the lambs to the crates before the faecal and urine samples could be measured for subsequent 7 days.

Feed refusals were measured before morning feeding at 0900 hours and experimental diets and refusals were weighed daily. The samples of experimental diets and refusals were obtained and placed in a freezer for subsequent chemical analysis. Total urine was also collected daily before morning feeding, in a 5 litre well labelled plastic container containing 20 ml of sulphuric acid 4M (H_2SO_4) to avoid nitrogen evaporation and bacterial infestation. A sample of urine (10%) or about 200 ml was transferred to 250 ml honey jar bottles, then frozen at -15 °C during the days of collection period for nitrogen retention analysis. Faecal matter was weighed and samples collected from the faecal bags in the morning of the collection period, to estimate the digestibility of dry matter (DM) and dietary constituents. Faecal samples (10%) were bulked for each animal with respect to their treatments during the collection period and stored at -15 °C before analysis. At the end of the experiment, urine samples were thawed and homogenized for preparation of a composite sample per lamb for quantification of urinary nitrogen. Faecal samples were oven dried at 135°C for 2 hours (DM; method 930.15, AOAC 2005), milled and sieved through 2 mm screen using Fritsch Pulverisette 16, (Idar-Oberstein, Germany) and analysed for various nutrients (see Section 5.2.5).

Apparent Nutrient digestibility (%) was calculated as the difference between nutrient intake and nutrient voided in the faeces divided by nutrient intake and the quotient multiplied by 100 (Marshal, 2001; Aduku, 2004; Okoruwa *et al*., 2012; Bello and Tsado, 2013).

Nitrogen balance (NB) was calculated from the amounts of N (g/d) consumed and excreted in the faeces and in the urine as follows: NB (g/d) = N-consumed – (N-faeces + N-urine).

Basal endogenous nitrogen (BEN) was calculated using the following equation by Agricultural and Food Research Council (AFRC) (1993):

BEN (g/d) = (0.35 + 0.018) x $BW^{0.75}$ ……………………..… Equation (1)

The value of Nitrogen retained (NR) was expressed as:

NR (g/d) = NB – BEN ………………………………………. Equation (2)

5.2.5 Analysis of the chemical composition of feed, urine and faecal samples

Samples of feeds and faeces were analysed using standard AOAC (2005) procedures to determine dry matter (DM; method 930.15), Ash (method 942.05), crude protein (CP; method 954.01) while organic matter percentage was calculated as 100%- % Ash. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to the methods of Van Soest *et al*. (1991) using an $ANKOM^{2000}$ Fibre Analyser (ANKOM Technology Corporation, NY, USA). Fat content in feed and faecal samples was determined using $ANKOM^{XT15}$ extractor, (ANKOM Technology Corporation, NY, USA), by boiling the samples in the die-ethyl ether for 60 minutes, then measuring the weight loss of samples after fat extraction then dividing it by initial sample weight multiplied by 100% to get percentage fat. Feed and faecal samples (1.25g) were digested using sulphuric acid–selenium (Se) digestion mixture following the procedure by Sahrawat *et al*. (2002) to determine Calcium (Ca) and phosphorus (P) levels. Inductively coupled plasma optical emission spectrometry

(ICP-OES) (Model Optima ™ 2100 DV, Perkin Elmer ® precisely, Germany) was used to read (Ca). Phosphorus was read using UV-Vis spectrophotometer (Model UV-1601, Shimadzu Corporation, Japan) and absorbance was determined at 670nm wavelength following Molybdenum blue method of Dickman and Bray (1940). The nitrogen content of the urine was determined by the Kjeldahl method according to AOAC (2005) procedure.

5.2.6 Data analysis

Growth performance, digestibility of dietary constituents and nitrogen balance data were subjected to analysis of variance (ANOVA) using general linear models (GLM) procedure of SAS (2002-2008) where parameters were arranged in a completely randomised design (CRD). Multiple comparisons of means were conducted using the least squares means separation (Ott, 1967) which was performed using the PDIFF option in GLM Procedure in SAS (2002-2008) to evaluate the significance and magnitude of the fixed effects at $P \leq 0.05$. Initial live weight was initially included as co-variate for final body weight but was found to have no effect, then it was omitted. The following statistical model was used:

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij}$$

Where;

$\boldsymbol{Y_{ij}}$ = response variable (feed intake and utilisation, nutrients digestibility and nitrogen balance of lambs fed MS, MSSS and SSS.

$\boldsymbol{\mu}$= population mean

$\boldsymbol{\beta_i}$= i^{th} treatment effect, i = (MS, MSSS and SSS).

$\boldsymbol{\varepsilon_{ij}}$ = random error $N(0, \sigma^2)$ (population mean, variance)

Time series data of weekly body weights were analysed using restricted maximum likelihood (REML) by using repeated measures within Proc Mixed procedure of SAS (2002-2008) to estimate variances and covariance (Holland, 2006). The model included the effect of treatment, time and treatment x time. Animal x treatment was used to specify variation between animals using the random statement. Unstructured (UN) form was selected as the most appropriate within-subject variance-covariance structure as its Akaike Information Criterion (AIC) and Huynh-Feldt (HF) were small compared to other structures.

5.3 Results

The initial weights of lambs did not have an influence on the final weights of lambs. There were no significant differences (P>0.05) between weekly weight means, measured for six weeks in growing Tswana lambs. However, an increase in total body weight of lambs fed various diets was recorded during the experiment as indicated by time effect (P<0.0001). Treatment x time interaction had no (P>0.05) effect on final weights. There was also weight gain recorded as time progressed in all the treatment though the gains were not significantly different (P>0.05).

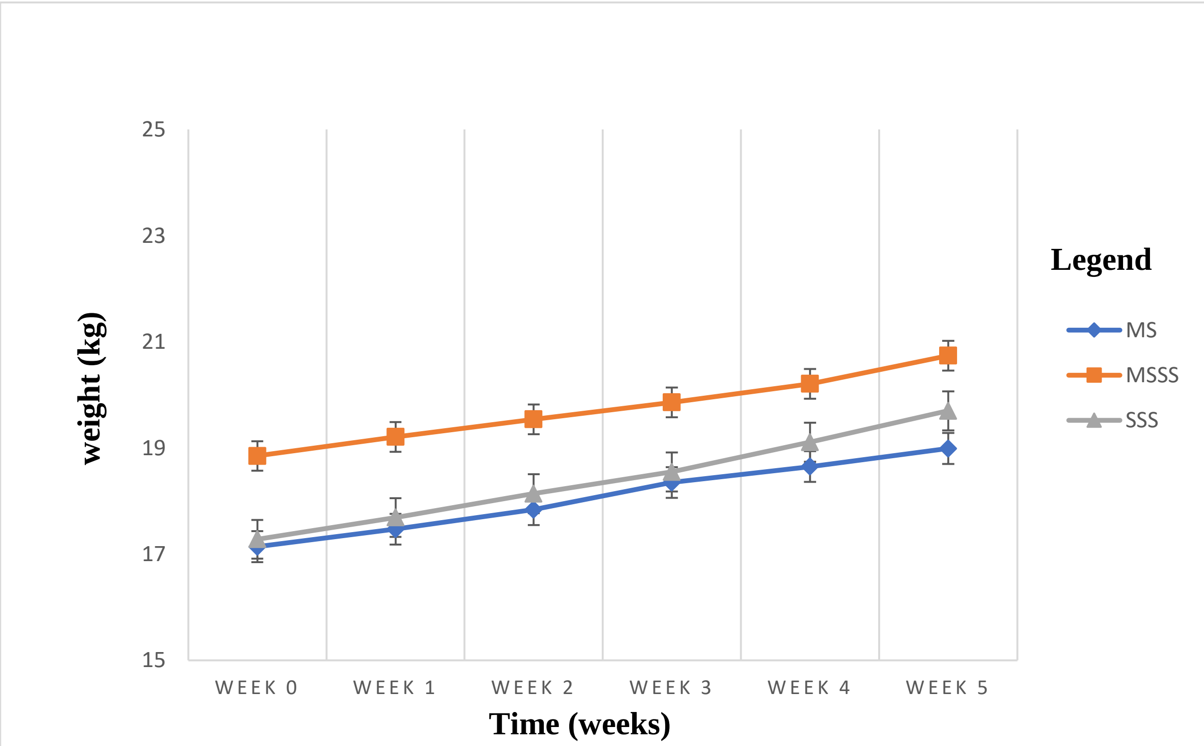

Figure 5.3.1. Weekly weights (kg) of growing Tswana lambs fed mushroom spent substrate and maize stover based-diets

The performance indices show that lambs that were fed SSS and MSSS had significantly higher ($P<0.05$) daily feed intake (DFI) of 586.00 g/day and 581.50 g/day respectively, which was different from that of lambs fed the control or MS diet (464.70 g/day). Feed conversion ratio (FCR), final body weight (FBW), total weight gain (TWG) and average daily gain (ADG) were the same across all lambs fed MS, MSSS and SSS diets.

Table 5.3.1. The effect of oyster mushroom spent substrate based-diets on performance parameters of growing Tswana lambs

	Experimental diets [1]				
Parameter- Evaluated [2]	MS (control)	MSSS	SSS	SEM	P value
IBW(kg)	17.10	18.80	17.30	1.46	0.6667
FBW(kg)	19.00	20.70	19.70	1.64	0.7630
DFI (g/day)	464.70^{b}	581.50^{a}	586.00^{a}	5.17	<0.0001
TWG (kg)	1.90	1.90	2.40	0.54	0.7202
ADG (g/day)	44.11	45.00	57.74	12.92	0.7152
FCR	14.68	17.11	12.34	4.47	0.7458

^{ab} Means in the same row, with the same superscripts, are not significantly different at ($P\geq0.05$).
Where; **SEM**= standard error of the mean
[1] **MS**= Maize stover based diet, **SSS**= Spent sorghum stover based diet, **MSSS**= Spent maize stover based diet.
[2] **IBW**= Initial body weight, **FBW =** Final body weight, **DFI**= Daily feed intake, **FCR**= Feed conversation ratio, **TWG**= Total weight gain, **ADG**= Average daily gain

Dry matter intake levels were significantly higher in MSSS fed lambs (547.08 g/day), followed by in lambs fed SSS (524.75 g/day) and lowest (446.71 g/day) in lambs fed the control diet. The lowest ash intake was observed in lambs that consumed MS (49.65 g/day) and MSSS (48.32 g/day) which was also not different between the groups compared to in lambs that were fed SSS (52.19 g/day). Moreover, ADL intake was the same in lambs fed MSSS (65.97 g/day) and SSS (67.21 g/day) but significantly lower ($P=0.0003$) than in lambs fed MS (76.21 g/day) diet.

Fat intake was significantly higher ($P<0.05$) in lambs fed SSS (81.78 g/day), followed by in lambs fed MSSS (57.80 g/day) and lowest in lambs fed MS (44.69 g/day). Intake of calcium and phosphorus were significantly higher in lambs fed SSS (24.31 and 11.48 g/day respectively). Tswana lambs that were fed MS (18.93 g/day) and MSSS (19.35 g/day) had similar calcium intakes which were however, lower ($P<0.0001$) than of those fed SSS (24.31 g/day). A similar pattern was observed for phosphorus in lambs fed MS (4.83 g/day) which had the lowest phosphorus intake. On the other hand, there were no significant differences ($P>0.05$) in NDF and ADF intakes observed between lambs offered experimental diets.

Table 5.3.2. The effect of oyster mushroom spent substrate based-diets on nutrient intake (g/day) of growing Tswana lambs

Parameters (g/day) [2]	Experimental diets [1]			SEM	P-value
	MS (control)	MSSS	SSS		
DM	446.71^{c}	547.08^{a}	524.75^{b}	5.48	<.0001
CP	70.60^{b}	91.11^{a}	91.69^{a}	0.69	<.0001
Ash	49.65^{b}	48.32^{b}	52.19^{a}	0.72	0.0126
NDF	78.34	82.84	84.03	4.51	0.6552
ADF	86.04	92.00	85.07	3.69	0.3938
ADL	76.21^{a}	65.97^{b}	67.21^{b}	1.16	0.0003
Fat	44.69^{c}	57.80^{b}	81.78^{a}	0.79	<.0001
Ca	18.93^{b}	19.35^{b}	24.31^{a}	0.44	<.0001
P	4.83^{c}	8.27^{b}	11.48^{a}	0.15	<.0001

[abc] Means in the same row, with the same superscripts, are not significantly different at ($P\geq0.05$).
Where: **SEM**= standard error of the mean
[1] **MS**= Maize stover based diet, **SSS**= Spent sorghum stover based diet, **MSSS**= Spent maize stover based diet
[2] **DM**= Dry matter, **CP**= Crude protein, **NDF**= Neutral detergent fibre, **ADF**= acid detergent fibre, **ADL**= acid detergent lignin, **Ca**= Calcium, **P**= Phosphorus

There was a treatment effect in nutrient digestibility for all parameters evaluated ranging from $P<0.0001$ to $P = 0.0442$ except for CP, Ash and NDF which were not different among lambs fed the three diets. Acid detergent fibre digestibility was significantly higher (51.82%) in lambs fed control diet and SSS (46.40 %) than in MSSS (44.50%). Digestibility of cell wall component (ADL) of the forage-based diets, was significantly higher in lambs fed MSSS (29.46%) and SSS (26.36%) and lowest in lambs fed MS (19.33%). Tswana lambs fed SSS had the highest fat digestibility (87.89%) while the lowest digestibility of fat was recorded in lambs fed MS (54.37%). There were significant differences in calcium disappearance in the three diets, with lambs fed SSS (84.00%) having significantly higher disappearance than MS (78.46%) and MSSS (72.94%). Similarly, phosphorus disappearance for MS (98.76%), MSSS (91.57%) and SSS (88.38%) were significantly different.

Table 5.3.3. The effect of oyster mushroom spent substrate based-diets on apparent nutrient digestibility (% DM) of growing Tswana lambs

	Experimental diets [1]				
Parameters (%) [2]	MS (control)	MSSS	SSS	SEM	P-value
DM	55.84^{a}	51.72^{b}	48.12^{b}	1.25	0.0060
CP	53.34	58.04	57.67	1.54	0.1097
Ash	18.52	21.31	29.51	3.07	0.0767
NDF	69.20	76.56	71.49	3.24	0.3066
ADF	51.82^{a}	44.50^{b}	46.40^{ab}	1.79	0.0442
ADL	19.33^{b}	29.46^{a}	26.36^{a}	1.51	0.0031
Fat	54.37^{c}	67.20^{b}	87.89^{a}	1.50	<.0001
Ca	78.46^{b}	72.94^{c}	84.00^{a}	0.51	<.0001
P	95.00^{a}	91.57^{b}	88.38^{c}	0.15	<.0001

[abc] Means in the same row, with the same superscripts, are not significantly different at ($P\geq0.05$).
Where: **SEM**= standard error of the mean
[1] **MS**= Maize stover based diet, **SSS**= Spent sorghum stover based diet, **MSSS**= Spent maize stover based diet
[2] **DM**= Dry matter, **CP**= Crude protein, **NDF**= Neutral detergent fibre, **ADF**= acid detergent fibre, **ADL**= acid detergent lignin, **Ca**= Calcium, **P**= Phosphorus

There was treatment effect in nitrogen intake and utilisation for all parameters measured ranging from P<.0001 to P=0.0044 except for urinary nitrogen (UN) and BEN which were not significantly different (P>0.05) among lambs fed diets. Lambs fed MSSS and SSS had significantly higher NI of 14.38 and 14.39 g/day respectively that those fed MS (11.15 g/day). Faecal nitrogen was high in lambs fed MSSS (1.95 g/day) than MS (1.82g/day) and SSS (1.70 g/day) which were similar. A positive NB was observed in all lamb's groups, with lambs fed MSSS and SSS having similar NB (P>0.05) of 12.23 and 12.50 g/day which was higher than of those lambs fed MS (9.14 g/day). Tswana lambs that consumed MSSS and SSS also retained similar nitrogen amounts of 8.80 and 9.06 g/day respectively which were significantly higher than of lambs fed MS (5.81 g/day). Faecal nitrogen was higher in lambs fed MSSS (1.95 g/day) and lowest in lambs fed MS (1.82 g/day) and SSS (1.70 g/day).

Table 5.3.4. The effect of oyster mushroom spent substrate based-diets on nitrogen intake and utilisation (g/day) of growing Tswana lambs

Parameters [2]	Experimental diets [1]			SEM	P-value
	MS (control)	MSSS	SSS		
NI	11.15^b	14.38^a	14.39^a	0.13	<.0001
FN	1.82^b	1.95^a	1.70^b	0.039	0.0044
UN	0.188	0.198	0.193	0.0019	0.1084
NB	9.14^b	12.23^a	12.50^a	0.13	<.0001
BEN	3.34	3.43	3.44	0.25	0.9480
NR	5.81^b	8.80^a	9.06^a	0.25	<.0001

[abc] Means in the same row, with the same superscripts, are not significantly different at (P≥0.05).
Where: **SEM**= Standard error of the mean
[1] **MS**= Maize stover based diet, **SSS**= Spent sorghum stover based diet, **MSSS**= Spent maize stover based diet
[2] **NI**= Nitrogen Intake, **FN**= Faecal nitrogen, **UN**= Urinary nitrogen, **NB**= Nitrogen balance, **BEN**= Basal endogenous nitrogen, **NR**= Nitrogen retention

5.4 Discussion

5.4.1 Body weight changes

There were no significant differences in the weekly weights of Tswana lambs fed MS, MSSS and SSSS diets over a six-week period with recorded final weight gains of 19.00 kg, 20.70 kg and 19.70 kg, respectively (Figure 5.3.1). This could be attributed to the lambs adapting to the newly formulated diets, which helps to establish a stable microbial population within the rumen. Usually, when grazing livestock are placed in feedlot diets/rations, performance goes down due to shifting in rumen bacteria community and fermentation dynamics (Fernando *et al*., 2010). With young animals being known to require more nutrients especially protein for fast growth (FAO, 2004) than older animals which was met in the present study, an expectation was that their weight gain will be rapid. This was not the case in the present study. More supply of protein may, however, lead to slow feed flow rate in the small intestine, with more heat being produced from the deamination process (Forbes, 1995). With deamination processes being energy demanding, it may decrease weight gain in the long run (Sudarman and Ito, 2000). Moreover, metabolisable energy concentration in the total dry matter (ME/DM) in the diets could have not been sufficient to sustain the utilisation of crude protein. Growing lambs weighing up to 20kg require ME of 11.70 MJ/kg (NRC, 1985) while the diets provided ME of 5.86-6.03 MJ/kg. Hence the effect of low ME/DM is on the efficiency of utilisation of metabolisable protein. This is one factor which has to be considered in the rationing of growing ruminants' energy requirement. The net energy for growth (NEg) could have not been met hence slow weight gains. According to Andrade *et al*. (2009), the rate of live weight gain has a significant impact on the NE content required for gains, with high weight gains triggering high-fat proportion produced. Yearling Tswana lambs genetically do not gain more weight rapidly and this could be one the reasons for their poor weight gains recorded in this study.

From the third week to the fifth week, there was an improved weight gain of MS (1.83 kg), MSSS (1.89kg) and SSS (2.43kg). Improved weight gain compared to that of the first two weeks indicates that the lambs were more adapted to the diets and turnover rate of rumen bacteria could have improved (Santra and Karim, 2003) hence, the efficiency of diet utilisation could have improved. There was no weight difference (P>0.05) in lambs fed treatments (MS-MSS, MS-SSS and MSSS-SSS) compared during the weeks assessed. The results indicate that even with extended feeding periods, it was likely that no lambs' group was going to be outstanding compared to others. Lack of differences in lambs' groups could be linked to minerals such as sulphur and certain amino acids from diets which promote microbial protein synthesis (Rodríguez *et al*., 2007) or rumen undegradable protein.

5.4.2 Feed intake and performance indices

In a study by Belewu and Popoola (2007), DFI was 327.67g/day for the control which did not contain spent substrate, 325.67 and 382.29 g/day for diets containing 20% and 25% *Rhizopus* treated sawdust fed to West African dwarf goats respectively. The DFI results of the current study are higher and this could be attributed to the palatability of the feed which improves feed intake especially MSSS and SSS which contained 55% spent sorghum substrate. According to Belewu and Popoola (2007), diets containing more fungi have increased feed intake and palatability.

It was observed that lambs that had high DFI (MSSS and SSS), produced the highest average daily gain (ADG) of 45.00 and 57.24 g/day respectively compared to the control diet though the differences were not significantly different. When feeding different levels of crude protein in total mixed rations to yearling Tswana, Legodimo (2013) observed high ADG ranging from 56.02 to 88.00 g/day as a result of high daily dry matter intake. Van Soest (1994) indicated that high feed intake usually promotes escape protein and microbial protein synthesis which provides protein absorbed in the small intestine of ruminant' animals. Microbial protein influences weight gains in ruminants. In the present study, ADG ranged from 44.11 to 57.24 g/day which is comparable with the results of Hwangbo (2014) who found ADG of 76.67 g/day in control diet and 71.11, 57.78 and 54.44 g/day for spent mushroom (*Flammuliua velutipes*) containing diets. Additionally, Kim *et al*. (2011) recorded ADG of 0.68kg/day when feeding weaned calves fermented oyster mushroom spent substrate compared to 0.63kg/day of the control. Higher ADG recorded by Hwangbo (2014) could be associated with improved FCR and high ruminal digestibility resulting in fatty acids and microbial protein being directly assimilated (Miszkiewicz *et al*., 2004).

Total weight gains (TWG) of 2.30 kg in the control diet and 2.13, 1.73 and 1.63 kg in spent mushroom (*Flammuliua velutipes*) containing diets were observed by Hwangbo (2014). The control results of the author are higher than of the current study. The control diet in a study by (Hwangbo, 2014) was composed of 50% concentrates and 50% rice straw compared to 45% concentrates and 55% maize straw used in the present study. Diets rich in concentrates normally leads to increased weight gains. This is because concentrates produce propionic acid which accounts for about 45% of acids required for weight gain (McDonald *et al*., 2011).

5.4.3 Digestibility of nutrients

In Study two, differences were observed due to forage type on chemical composition and fermentative dynamics of spend mushroom substrates-based diets. The fact that *in vivo* nutrient digestibility was significantly different between different cereal spent substrate based-diets for dry matter (DM), acid detergent fibre (ADF), acid detergent lignin (ADL), fat, calcium (Ca) and phosphorus (P) is telling in that even after treating, inherent variation between cereal stover remains.

There was an inversely proportional pattern observed in dry matter digestibility and intake. i.e., the lower the intake level, the higher the digestibility of the diet. Dry matter digestibility of the present study was higher than that of Sarker *et al*. (2016). In another study, Xu *et al*. (2010) observed DM digestibility ranging from 64.7 to 68.5% in complete diets containing 6.5 and 13.0% of the spent mushroom substrate respectively while 69.9 % DM digestibility was observed in the control diet. The findings from that study contradict with the present study and differences between % DM digestibility could probably explain the accumulation of different nutrients in the diets. Van Soest (1994) stated that DM digestibility and subsequently other dietary nutrients are affected by the proportion of cell wall contents and their availability for digestion which in turn are determined by the extent of lignification and other factors. Therefore, the DM digestibility of the present study suggest that most of the dietary constituents in lambs fed MS diet will be available for fermentation by amylolytic bacteria and protozoa in the rumen compared to nutrients in MSSS and SSS diets, hence high nutrients uptake by lambs.

In the present study, ash digestibility remained unchanged between lambs fed MS, MSSS and SSS diets but there was a tendency P =0.0767 for high ash digestibility in SSS. Ash digestibility of lambs fed experimental diets in the current study ranged from 18.52-29.51 % which was marginally close to that reported by Seok *et al*. (2016) when feeding sheep complete diet containing by-product fed (BF) silage and 45 % spent mushroom substrate. Percentage ash digestibility coefficients of 28.7 % were observed in the BF silage with 45 % spent mushroom substrate respectively. According to Okoruwa and Njidda (2012), the level at which nutrients are digested in diets mainly depends on factors such as variation in nutrient composition of the diet consumed by an animal and differential level of the diet. McDonald *et al*. (2011) highlighted that ash represents the inorganic components of the diet. Improved ash digestibility in all experimental diets indicates high degradability or availability of the mineral constituents.

Lambs fed the diets will not be deprived of mineral elements and their general health will be improved.

Crude protein digestibility was the same across lambs fed experimental diets. Digestibility levels of 53.34, 58.04 and 57.67 % were observed in lambs fed MS, MSSS and SSS diets respectively. This finding could explain the lack of differences in weight gains among lambs offered the diets. The CP digestibility findings are inconsistent with of Seok *et al*. (2016) who observed improved digestibility of CP (from 68.8 to 70.1 %) after feeding sheep by-product feed (BF)-based silage containing 45% spent mushroom substrate compared to the control (concentrate mix + rye straw). Lower CP digestibility reported in the present study could be due to fermentable energy which could have not been enough to capture the excess nitrogen hence no synergy between nitrogen and energy. Moreover, low CP digestibility could be associated with intake levels and concentration of crude protein nutrient in the consumed diets or lack of rumen-protected protein. Intake of CP ranged from 97.9 to 141.3 g/day in the study by Seok *et al*. (2016) while of the present study ranged from 70.60 to 91.69 g/day. Moreover, there could be a possibility of some protein being bound to lignin (lignin bound protein) hence not being accessible to rumen microbes for digestion (Santos *et al*., 2012). This could have led to low CP digestibility in the present study when compared to that of the study by Seok *et al*. (2016). Low digestibility of protein leads to its reduced availability to the animal hence poor animal performance as significant weight gains will not be achieved.

Hwangbo (2014) observed high digestibility coefficients of ADF (42.83-48.97 vs 45.14 %), NDF (52.77-57.76 vs 53.77 %) and fat (67.47-69.95 vs 70.08 %) in total mixed rations containing spent mushroom (*Flammuliua velutipes*) vs the control diet respectively fed to Korean Black Goats. The ADF findings of the author are comparable with of the present study (ADF;44.50-46.40 %), while NDF contents reported by (Hwangbo, 2014) were lower than of the present study (76.56-71.49 %) in complete diets containing spent mushroom substrates.

The results suggest an improved energy proportion which could be useful in rumen fermentation hence providing a good balance of absorbed nutrients by the animal (Yashim *et al*., 2016). Fat digestibility was high (67.20-87.89 %) in the present study when compared to of reported by Hwangbo (2014). High fat digestibility recorded in the current study indicates that the spent mushroom substrates based-diets have the capacity to improve the utilization of ether extract. In another study, Sarker *et al*. (2016) observed ADF digestibility % of 47.24 and 45.66, NDF digestibility % of 49.37 and 52.05 and fat digestibility of 30.45 and 34.92 % when feeding beef cattle complete diets based on plain rice straw compared to rice straw spent mushroom substrate respectively. The findings of Sarker *et al*. (2016) are comparable to of the present study. The results of the present study indicate that mushroom treatment exposed cell wall components to enzyme degradation during mycelium growth hence high digestibility of fibre. This confirms the assertions made by Fazaeli and Masoodi (2006); Phan and Sabaratnam (2012) stated that fungi improve ruminal digestion of fibre. Improved fibre digestibility (ADF and NDF) in treated diets means that diets will have improved feeding value and nutrient supply. This will increase the availability of fermentable energy to ruminal microbes (Mahesh and Mohini, 2013) thus improving the growth rate in indigenous lambs.

With regard to minerals, the availability of phosphorus was improved in all experimental diets. Phosphorus disappearance was 95.00, 91.57 and 88.38 % in lambs fed MS, MSSS and SSS diets respectively. Conversely, disappearance of Ca was high in lambs fed SSS (84.00 %) and low in lambs fed MSSS (72.94 %) diet. In communal grazing systems like of Botswana, reports on the occurrence of Ca and P animal deficiency has been made (APRU, 1980), with phosphorus being one of the most limiting nutrients in pasture production. The results of the present study show that feeding all diets can supply Ca and P to animals as these minerals highly disappear in all the tested diets. The experimental diets have the potential of meeting the 1: 1 to 2: 1 calcium and phosphorus requirement of grazing ruminants (McDonald *et al*., 2011).

5.4.4 Nitrogen utilisation and balance

The nitrogen metabolism results indicated variations in nitrogen intake (g/day) across the dietary treatments. Nitrogen intake levels were high in lambs that consumed SSS and MSSS diets, (14.39 and 14.38 g/day) respectively. Hwangbo (2014) reported NI levels of 9.10 g/day in control diet and 10.43-11.49 g/day in spent mushroom containing diets, which were lower than that of the present study. The difference could be associated with varying amounts of dry matter intake which in turn would be influenced by CP levels of the diets. In a study by Legodimo (2013), total mixed rations containing low protein levels produced high feed intake, while Allen (2000) observed an exponential decline in dry matter intake when CP increased in lactating dairy cows. Hence CP levels in the current study could have permitted high dry matter intake compared to the other studies thus increased NI.

The lambs on MSSS had the highest (1.95 g/day) faecal nitrogen when compared to of the other diets. High FN in lambs fed MSSS implies that most of the nitrogen was not fully utilised and indigestible hence being excreted. According to Santos *et al*. (2012), the nitrogen could be bound to the cell wall component, ADL hence being excreted. In some instances, high FN could be a result of endogenous nitrogen which is often mobilised from endogenous sources such as the gut walls and excreted (McDonald *et al*., 2011). Detmann *et al*. (2014) stated that metabolisable protein could be achieved through provision or inclusion of rumen undegradable protein (RUP) which can be recycled and increase nitrogen availability in the rumen resulting in increased microbial protein. By including RUP in diets, the sources of nitrogen such as urea will have to be reduced to avoid nitrogen wastage through proteolysis processes (Detmann *et* *al*., 2014). When substituting Timothy hay with a spent mushroom substrate in total mixed ration silages, Xu *et al*. (2010) recorded an increase in FN of 6.28 to 7.36 g/day. The high faecal nitrogen excreted in the study by Xu *et al*. (2010) and MSSS in the present study could also be linked to the presence of condensed tannins (CT) which were however similar between MSSS and SSS. Besides, maize is not known for having CT, especially in the stalk. Anti-nutritional factors such as CT hinder protein absorption in the small intestines (Kim *et al*., 1996). Kumar and Vaithiyanathan (1990) stated that anti-nutritional compounds such as tannins, forms complexes with mucoprotein secreted from the gut wall which tends to inhibit protein degradation in the rumen and caecal and this leads to increase in FN. Tannins levels reported in chapter 4; Table 4.3.2 were low and not significant among experimental diets and their effect in protein degradability could have been insignificant.

Treatment did not affect urinary nitrogen. Urinary nitrogen (UN) was 0.188, 0.198 and 0.193 g/day for MS, MSSS and SSS fed lambs respectively. Urinary nitrogen losses of 2.47 g/day and 3.08-3.17 g/day were reported by Hwangbo (2014) when feeding the control; not containing any spent substrate and spent mushroom containing diets respectively. With different amounts and types of protein being used in the present study compared to that of Hwangbo *et al*. (2014), the protein metabolic pathways may have been different hence different urinary losses recorded. Decandia *et al*. (2011) indicated that UN in goats and sheep is strongly correlated to dietary CP levels, with UN increasing when protein supplementation increases.

In all the experimental lambs', a positive nitrogen balance (NB) was recorded indicating that all experimental diets supplied the required N amounts to the lambs. However, lambs on MSSS and SSS had high NB of 12.23 and 12.50 g/day respectively. The apparent nitrogen balance was directly proportional to the NI as NB increased with increase in nitrogen intake. These nitrogen balance statuses could mean the nitrogen sources in the experimental diets provided the right amount and quality for the lambs to metabolise. Moreover, the positive NB could be supported by an increase in weight gains which were recorded. Even though the diets were formulated to be iso-nitrogenous and their CP intake was the same, the metabolic pathways through which the protein deposition in the body could have been different which led to variations in nitrogen utilisation. Babu *et al*. (2014) reported positive N balance in Nellore lambs fed sweet sorghum stover based complete ration. The findings of the present study are also in accordance with that of Knowlton *et al*. (2002) who reported no significant differences in nitrogen balance in lactating cows fed total mixed ration (TMR) containing 45% and 61 % forage. The results suggest that nitrogen was available for metabolism which could be associated with adequate protein supply. In the tropical environments like of Botswana, protein supplementation is common during the dry periods, but CP supplementation could hinder the efficiency at which metabolisable protein is converted into net protein (Costa *et al*., 2011). Low nitrogen amounts were retained, consequently resulting in low weight gains, whereas there was high NB in MSSS and SSS diets. The protein content of the diets was equal to the daily CP requirement for a growing lamb with an average body weight of 17-20 kg. Additionally, the NI was adequate to support an ADG of 100 g which was targeted.

Basal endogenous nitrogen (BEN) indicates the demand for metabolisable protein, which is used for maintenance with an efficiency of 1.0 (McDonald *et al*., 2011). BEN was 3.34, 3.43 and 3.44 g/day for lambs fed diets MS, MSSS and SSS respectively. It did not show varied response due to the incorporation of spent mushroom substrates in complete diets. The endogenous nitrogen per unit weight gain could be predicted to be the same in all the experimental lambs. With the same metabolisable protein demands in all lambs, it means the diets supplied the same nutrients quantity which would be deposited for renewal of tissue protein. In order to confirm this, tests on NR were conducted to measure the efficiency of utilisation of available nitrogen for gain.

Most of the nitrogen was retained in lambs fed MSSS and SSS compared to those on MS diet. One could have expected more weight gains from the lambs offered MSSS and SSS diets since they had high NR which the lambs could have retained as lean protein. In a study by Hwangbo (2014), nitrogen retained was 3.72 g/day in control diet which did not contain spent mushroom substrates while SMS containing based-diets it ranged from 3.65-4.89 g/day. The differences could have been due to crude protein levels and intake of the experimental diets. Crude protein levels of the diets ranged between 10.93-10.97 % in the study by Hwangbo (2014) which was lower than that of the present study. Aye and Adegun (2010) stated that the NR for high protein diets is always higher compared to low protein level diets. High NR could suggest an improved utilization of the nitrogen absorbed which was not the case in the present study. Maybe the indigenous lambs reached their genetic potential for growth hence low weight gains

5.5 Conclusions

There were no significant differences in the growth performance of Tswana lambs fed MS (control) MSSS and SSS. However, performance indices revealed high DFI in lambs fed SSS and MSSS compared to MS. Other evaluated parameters such as IBW, TWG, ADG and FCR were similar in all lambs offered the diets. Differences were observed in nutrient digestibility parameters except for CP, ash and NDF. Growing Tswana lambs that were fed SSS diet had the highest ADF digestibility which was the same as in MS. Tswana lambs fed SSS had the highest ADL, Fat and Ca digestibility indicating an improved availability of nutrients when compared to the diets. Highest DM and phosphorus digestibility was recorded in MS diet.

Growing Tswana lambs that were fed MS, MSSS and SSS had the same UN and BEN. Faecal nitrogen was high in lambs that consumed MSSS, with MS and SSS having the same FN amounts, meaning that nitrogen was not utilised efficiently in the rumen or not absorbed in the small intestine hence being excreted in faeces. However, NI was high in lambs on MSSS and SSS. This gave rise to high NB and N retention observed. Feeding mushroom spent substrate based-diets did not support better growth performance or weight gains than the control but increased feed intake and digestibility of nutrients such as ADL, fat and Ca disappearance.

5.6 Recommendations

Feeding sorghum and maize spent substrate based-diet to growing Tswana lambs will improve feed intake and nutrient digestibility. However, farmers, retail shops and livestock enterprises should understand the type of protein included in formulated diets, its utilisation and how it can help in more production of microbial protein as these will help to efficiently utilise feed resources in the tropics and achieve improved weight gains in growing lambs. Perhaps high quality protected protein such as fish meal or fatty acid coated protein that escapes rumen degradation is needed in small amounts to promote desired growth.

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CHAPTER SIX

6.0 General conclusions and future research

6.1. General conclusion

The first study (chapter three) revealed the current uses and disposal of cereal crop residues in Kgatleng, Kweneng and Southern districts of Botswana. The data collected would be important to farmers and extension officers as it will change their current perceptions on the value of crop residues as livestock feed resource. Extension officer could also be able to advise farmers accordingly. Those farmers who leave their crop residues to be trampled by animals because they think they are of low value, will find it worth to cut and carry these crop residues to their homesteads have a choice of improving their nutritive content through mixing them with other feed ingredients or upgrade them with either urea ammonisation, wood ash treatment or silage making. The goal to utilise the crop residues as animal feed will be greatly enriched. It was found out that most farmers harvested and stored their crop residues for later use. Sorghum, millet and maize are commonly available in all the three districts surveyed. All classes of animals (98.4%) were offered cereal crop residues, with only 1.6% being calves. This shows that farmers know that calves have less developed rumen hence they will not utilise the crop residues effectively. Maize stover was perceived to be of very poor quality by 54% of interviewed farmers, with sorghum stover being rated better and millet' quality not being known. Farmers in all districts tried to improve the quality of their harvested residues through the addition of other feedstuffs such as salt, molasses, wheat bran, lablab and di-calcium phosphate. With protein sources such as lablab being expensive, some farmers supplemented these cereal crop residues with cowpeas straw and this is expected to increase the supply of nitrogen which is needed for rumen microbes and help in improving the digestibility of millet, sorghum and maize stovers in ruminants. Conversely, 95.2% of the farmers agreed with the suggested idea of using crop residues for mushroom production in order to improve their utilisation by livestock. This would help them make additional income (through selling mushrooms), improved human nutrition (they can also consume mushrooms at family level) improve nutrition of livestock kept by small-scale farmers (as spent substrates from mushroom cultivation have reduced fibre levels hence high intakes compared to raw straw) when they adopt mushroom production as part of a cereal-livestock production system.

Processing of crop residues through treatment with fungi may improve their digestibility and intake by ruminant animals. In an earlier study, in our laboratory, it has shown that spent mushroom substrates from cereal crop residues have reduced fibre levels, it is expected digestibility will be increased. Spent mushroom substrates can then be mixed with other feed ingredients to make complete rations. To find out the potential of crop residues in ruminant feeding, one of the objectives of this project was to determine the effect of crop residue type on the nutritional composition, the *in vitro* and *in sacco* digestibility of oyster mushroom spent substrate based-diets for growing indigenous lambs. It was found out that cereal stover based-diets had crude protein levels ranging from 15.09 to 15.92% which could meet the growth requirement of growing lambs. Moreover, spent mushroom substrate based-diets had reduced fibre levels ADL (6.16-9.04%) and ADF (14.52-17.30%) compared to original stover based diets which had ADL (7.46-10.59%) and ADF (17.41-18.42%). The reduced fibre levels will lead to improved feed intake by livestock. The quality of spent substrate based-diets investigated was high as indexed by high dry matter digestibility, with calcium (Ca) and phosphorus (P) levels increasing in treated cereal crop residues based diets. This could meet Ca and P requirements of grazing animals. Treated diets had high soluble fractions and effective degradability at both outflow rates compared to the untreated ones. These improved degradability parameters, meaning better nutrient availability and utilisation by livestock. Farmers could then adopt mushroom production into their crop production system, therefore reducing the dietary supplementation in the late dry season as treated crop residues have high nutrients content.

Production of complete diets or total mixed rations (TMRs) based on cereal crop residues for sheep may help to meet feed demands during the dry season hence supplying nutrients that are required by livestock for production, as the amount of pastures and quality are severely reduced. The utilisation of local crop residues might also reduce the feed costs in farming as feeds importation will be reduced. Lamb production may increase locally, reducing its importation. Farmers can realise improved income after selling lambs fed complete diets based on local crop residues. To discover the potential of complete rations in improving better growth performance in indigenous lambs, one of the objectives on animal performance study was designed. It was found out that indigenous lambs fed sorghum spent substrate based-diet (SSS) had the highest daily feed intake (DFI) of 586.00 g/day which was the same as of those fed MSSS (581.50 g/day) with both being higher than of lambs on MS (464.70 g/day). Feed intake translated into the same average daily gains of 44.11, 45.00 and 57.24 g/day across lambs fed MS, MSSS and SSS respectively

The improved nutrient digestibility of ADF which was the same as in MS, ADL which was also the same as in MSSS, fat and Ca in lambs fed SSS could mean farmers can use more sorghum substrate in mushroom production as it could be utilised well and supply more nutrients when included in complete rations compared to other cereal stovers.

Nitrogen intake and metabolism were different across lambs in different diets groups. MSSS and SSS diets had the highest nitrogen intake of 14.38 and 14.39 g/day respectively while lambs on MS had the lowest N intake 11.15 g/day. A positive nitrogen balance was recorded in all the experimental lambs. This shows that all experimental diets provided or met the required nitrogen of the experimental lambs. Nitrogen balance in lambs on MSSS (12.23 g/day) and

SSS (12.50 g/day) were significantly higher than those of lambs fed MS 9.14 g/day). This might be as a result of higher nitrogen intake by lambs on MSSS and SSS diets. Nitrogen retained was high in lambs fed MSSS and SSS, 8.80 and 9.06 g/day respectively than in lambs fed MS (5.81 g/day). The results from the present study indicate that maize and sorghum stover which is cheaper and unexploited source of roughage could be used in mushroom production to acquire spent mushroom substrates. Then they could be used to formulate complete diets which have shown to improve the feed intake and digestibility of ADF, ADL, fat and calcium.

6.2 Future Research

Improved nutritive value of crop residues through white-rot fungi treatment shows that there are possibilities of developing technologies aimed at only identifying and isolating and specific and highly ligninolytic fungus in nature which can be cultivated and used in the commercial mass production of ligninase enzymes. If that is possible, caution should then be practised, making sure that only lignin fraction of forage is degraded and carbohydrates structures are not destroyed. This can only be achieved through genetic manipulation of ligninolytic fungus.

The inclusion of non-degradable protein in diets with high rumen bypass and be fully utilised in the small intestine of the lambs needs to be explored. This will help in digestion of protein to form limiting amino acids and peptides resulting in better utilisation of proteins in the body and more nutritive feed at a cheaper price.

APPENDICES

RESEARCH QUESTIONNAIRE

VALUE-ADDITION OF CEREAL CROP RESIDUES FOR IMPROVING THE INCOME, NUTRITION AND SMALL-STOCK FEED FOR SMALL-SCALE FARMERS USING LOW TECHNOLOGY OYSTER MUSHROOM PRODUCTION

SECTION A

BIOGRAPHIC DATA / CHARACTERISTICS OF THE RESPONDENTS

1. Name of Household Head

____

2. Location (Village and District):

____

3. Gender of Household Head 1. Female [ ] 2. Male [ ]

4. Age Range (Years):

1. Less than 21 [ ] 2. 21-25 [ ] 3. 26-30 [ ] 4. 31-35 [ ] 5. 36-40 [ ] 6. 41-45 [ ] 7. 46-50 [ ] 8. 51-55 [ ] 9. 56-60 [ ] 10. 61-65 [ ] 11. More than 65 [ ]

5. Marital Status of Household Head: 1. Single [ ] 2. Married [ ] 3. Divorced [ ] 4. Separated [ ] 5. Cohabiting [ ] 6. Widowed [ ]

6. Size (members) of the Farm Household

6.1 Number of people in the household 1[ ] 2[ ] 3[ ] 4[ ] 5[ ] 6 [ ] 7 [6.2 Number of females in the household 1[ ] 2[ ] 3[ ] 4[ ] 5[ ] 6 [ ] 7 [ ]

6.3 Number of income earners in household 1[ ] 2[ ] 3[ ] 4[ ] 5[ ] 6 [ ] 7 [6.4 Number of female income earners in household 1[ ] 2[ ] 3[ ] 4[ ] 5[ ] 6 [ ] 7 [ ]

7. Highest Educational Level Attained by Head of Household

1. Primary School [ ] 2. Secondary (Junior) [ ] 3. Secondary (Senior) [ ] 4. College Certificate [ ]

5. College Diploma [ ] 6. College Degree [ ] 7. Masters [ ] 8. Doctorate

[ ] 9. Other (specify)

____

SECTION B

FARM / FARMING STATUS

10. What is the size of the field / farm (hectares)? ____________ hectares.

11. Describe possession status of the field / farm. 1. Owned [ ] 2. Rented [ ] 3. Leased [ ]

12. How would you describe your farming practice? 1. Full Time [ ] 2. Part Time [ ]

13. If you do farming on part time basis, what economic activity are you involved in on full time basis?

1. Not economically active [ ] 2. Non-agriculture own business (state type ____________________) [ ]
3. Paid employment [ ] 4. Unpaid family employment [ ] 5. Other (Specify): ______________

14. How long have you practiced farming (years)?
Less than 1 year [ ] 1 [ ] 2[ ] 3[ ] 4[ ] 5 [ ] More than 5 years [ ]

15. What is the main economic activity you were doing before you started farming? 1. Not economically active [ ] 2. Non-agriculture own business (state type ____________________) [ ] 3. Paid employment [ ] 4. Unpaid family employment [ ] 5. Other (Specify): ______________

SECTION C

FARMING SYSTEM

16. Which of the following planting methods do you practice? (Please indicate ONLY ONE choice).

1. Row planting only [ ]   2. Broadcasting only [ ]   3. Both row planting and broadcasting [ ]

If your answer is [1], skip to question 18.

If your answer is [3], proceed with question 17.

17. Specify how much area of land was under each planting method:

Area row planted __________ hectares.

Area Broadcasted __________ hectares.

18. What cereal crops do you grow?

1. Maize [ ]   2. Sorghum [ ]   3. Millet [ ]

19. What grain legumes do you grow?

1. Cowpeas [ ]   2. Groundnuts [ ] 3. Bambara groundnuts [ ]

SECTION D

GRAIN YIELD AND ITS DISPOSAL

20. Please provide a few details below on production, consumption and marketing of grain crops you grow.

Crop	Area Planted (Ha)	Yield/Ha (No. of 50kg bags per ha)	Proportion of grain consumed at home (No. of 50kg bags)	Proportion of grain sold (No. of 50kg bags)	Unit Price (Average price per 50kg bag)
Maize					
Sorghum					
Millet					
Cowpeas					
Groundnuts					
Bambara groundnuts					

SECTION E

CEREAL CROP RESIDUES AND THEIR DISPOSAL

21. What happens to cereal crop residues (stalks / stover) after harvest of grain?

1. Harvested and stored for feeding animals [ ] 2. Harvested and sold as animal feed [ ] 3. Left in field to be grazed by animals [ ] 4. Ploughed back into the soil as manure [ ] 5. Left on surface as part of conservation tillage [ ] 6. Just cleared and burnt in field [ ]

22. If your answer to Question 21 is [1], please specify the species and class of animals you feed with the cereal crop residues (stalks / stover):

Species of animals	**Class** of animals

23. If harvested, do you weigh the cereal crop residues? 1. Yes [ ] 2. No [ ]

24. If residues are weighed, please provide a few details below on production, use and marketing of the cereal crop residues from your field.

Crop	Crop residue /Ha (No. of 50kg bags per hectare)	Proportion of crop residue used at home (No. of 50kg bags)	Proportion of crop residue sold (No. of 50kg bags)	Unit Price (Average price per 50kg bag of crop residue)
Maize				
Sorghum				
Millet				

25. How would you rate the nutritional value (quality) of the cereal crop residues from your field? Please place only one tick (√) under each "crop residue column" to indicate your perceived quality score.

Perceived Quality Score	Maize residue	Sorghum residue	Millet residue
1. Very low quality			
2. Low quality			
3. I don't know			
4. High quality			
5. Very high quality			

26. If your answer to Question 25 is [1] or [2], please specify what you do (or you may do) to improve the quality of cereal crop residues if there are of low quality.

____

____

27. How likely would it be for you to participate in a study that aims at improving the nutritional value of cereal crop residues by growing mushrooms in your field?

1. Very Unlikely [ ] 2. Unlikely [ ] 3. Undecided [ ] 4. Likely [ ] 5. Very Likely [ ]

SECTION F

LEGUME CROP RESIDUES AND THEIR DISPOSAL

28. What happens to legume crop residues / stalks after harvest of grain?

1. Harvested and stored for feeding animals [ ] 2. Harvested and sold as animal feed [ ] 3. Left in field to be grazed by animals [ ] 4. Ploughed back into the soil as manure [ ] 5. Left on surface as part of conservation tillage [ ] 6. Just cleared and burnt in field [ ]

29. If your answer to Question 28 is [1], please specify the species and class of animals you feed with the legume crop residues / stalks:

Species of animals	**Class** of animals

30. If harvested, do you weigh the legume crop residues?1. Yes [ ] 2. No [ ]

31. If residues are weighed, please provide a few details below on production, use and marketing of the legume crop residues from your field.

Crop	Crop residue /Ha (No. of 50kg bags per hectare)	Proportion of crop residue used at home (No. of 50kg bags)	Proportion of crop residue sold (No. of 50kg bags)	Unit Price (Average price per 50kg bag of crop residue)
Cowpeas				
Groundnuts				
Bambara groundnuts				

32. How would you rate the nutritional value (quality) of the legume crop residues from your field? Please place only one tick (√) under each "crop residue column" to indicate your perceived quality score.

Perceived Quality	Cowpeas residue	Groundnuts	Bambara groundnuts
1. Very low quality			
2. Low quality			
3. I don't know			
4. High quality			
5. Very high quality			

33. If your answer to Question 32 is [1] or [2], please specify what you do (or you may do) to improve the quality of legume crop residues if there are of low quality.

____

____

34. How likely would it be for you to participate in a study that aims at improving the nutritional value of legume crop residues by growing mushrooms in your field?

1. Very Unlikely [ ] 2. Unlikely [ ] 3. Undecided [ ] 4. Likely [ ] 5. Very Likely [ ]

SECTION G

FARMERS' PERCEPTIONS OF MUSHROOMS AND ATTITUDE TOWARDS MUSHROOM PRODUCTION

35. Do you know mushrooms? 1. Yes [ ] 2. No [ ]

If your answer to Question 35 is NO, skip to question 41. Otherwise proceed to question 36.

36. If your answer to Question 35 is YES, please indicate which mushrooms you know:

I know mushrooms that grow naturally in the bush / wild 1. Yes [ ] 2. No [ ]

I know mushrooms that are grown/cultivated in the fields 1. Yes [ ] 2. No [ ]

37. Do you eat mushrooms? 1. Yes [ ] 2. No [ ]

If your answer to Question 37 is YES, skip to question 39. Otherwise proceed to question 38.

38. If your answer to Question 37 is NO, please state reason why you do not eat mushrooms

____

____

39. If your answer to Question 37 is YES, which type of mushrooms do you eat?

____

____

40. If your answer to Question 37 is YES, where do you find the mushrooms that you eat?

____

____

41. Would you be interested in growing mushrooms for the following reasons:

To improve your family's nutritional status 1. Yes [ ] 2. No [ ]

To improve your livestock feed's nutritional status 1. Yes [ ] 2. No [ ]

To improve soil fertility in your field 1. Yes [ ] 2. No [ ]

To generate extra income for your household1. Yes [ ] 2. No [ ]

END OF QUESTIONNAIRE. THANK YOU FOR YOUR CONTRIBUTION AND

WILLINGNESS TO PARTICIPATE IN THIS SURVEY