



**The Potential of *Morama* Bean Cake (*Tylosema esculentum*) as an
Alternative Protein Source for Ruminants**

By

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DECLARATION

I, John Kenneth Mthetho, declare that the thesis submitted for the award of Doctor of Philosophy (PhD) in Animal Science at the Botswana University of Agriculture and Natural Resources (BUAN) is my own independent work and has not previously been submitted to this or any other university. Specific contributions by others are duly acknowledged.

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DEDICATION

This work is a dedication to my wife and children who lost marital and paternal care during my absence during the challenging days of my laboratory work at the university.

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LIST OF ABBREVIATIONS

<i>a</i>	Rapid soluble fraction
ADF	Acid detergent fibre
ADL	Acid detergent lignin
<i>b</i>	Slowly degradable fraction
BAMB	Botswana Agricultural Marketing Board
BW	Bag weight
CP	Crude protein
CPD	Crude protein degradability
CRD	Completely randomized design
CT	Condensed tannin
CPD	Crude protein degradation
dCP	Degradable fractions of crude protein
dDM	Degradable fractions of dry matter
DM	Dry matter
DMD	Dry matter degradability
ED	Effective degradability
ED _{CP}	Effective degradability of crude protein

ED _{DM}	Effective degradability of dry matter
ED _{NDF}	Effective degradability of neutral detergent fibre
EE	Ether extract
<i>h</i>	Hour
<i>k_d</i>	Rate of degradation
<i>k_p</i>	Rate of passage/outflow rate
MBCD	Moramba bean cake decorticated
ME	Metabolizable energy
NDF	Neutral detergent fibre
NFC	Non-fibre carbohydrate
OM	Organic matter
P _v	Probability value
RFD _{CP}	Rumen fluid disappearance of crude protein
RFD _{DM}	Rumen fluid disappearance of dry matter
RFD _{NDF}	Rumen fluid disappearance of neutral detergent fibre
RMBC	Roasted moramba bean cake
<i>u</i>	Undegradable residual
U _{CP}	Undegradable crude protein

U _{DM}	Undegradable dry matter
WMBC	Whole morama bean cake
WSSC	Whole sunflower seedcake

GENERAL ABSTRACT

The biodiversity of plants in Botswana offers great opportunities for the development of ailing ruminant livestock farming. The qualitative and quantitative inadequacy of rangeland is the biggest challenge to livestock production in Botswana. Poor and rather irregular distribution of precipitation worsens the situation. However, pastures in these ecosystems harbour underutilized legumes that are endowed with essential nutrients. One of the potential protein sources that could improve ruminant nutrition is *Morama* bean cake (*Tylosema esculentum*). The nuts are often eaten raw or roasted by people who live where the plants are found, often in dry areas of the Kalahari Desert.

To assess their potential in ruminant feeding, *Morama* beans were prepared using three distinct methods: roasted *Morama* bean cake (RMBC), whole *Morama* bean cake (WMBC), and decorticated *Morama* bean cake (MBCD). As feed formulations for ruminants typically include hulled sunflower seed cake (WSSC), a sunflower-based control diet was similarly prepared using hulled sunflower seed cake.

The first study (comprising the first and second experiments) compared the differences between the nutritional attributes of the different *Morama* and sunflower seed cakes, as well as the *in vitro* DM and CP and *in situ* DM and CP rumen degradation properties. Therefore, the study objectives were achieved through laboratory methods (wet chemistry or close-up analysis and *in vitro* studies) and animal experiments (*in situ*). The techniques determined the nutritional values and potential rumen breakdown of the forage.

The *in vitro* and *in situ* results for *Morama* and sunflower seed cakes correlated in many ways, but the *in vitro* method appeared to significantly underestimate degradation rates compared to the *in situ* method. In this case, *in vitro* findings suggested that the crude proteins for WMBC and RMBC were less degradable in rumen fluid than MBCD and WSSC ($P < 0.05$). The CP disappearance values of WSSC and MBCD had reached 90% and 97.2%, respectively, within 24 hours of incubation. RMBC generally exhibited higher values for the *a* and *b* fractions, indicating a higher potential for CP degradation in the rumen. These findings underscore the importance of selecting appropriate feed treatments to optimize CP degradation and effective degradability in ruminants, ultimately enhancing nutrient utilization and animal performance.

The second study objective was to compare the protein absorption capacity of roasted *Morama* bean cake and sunflower seed cake on a basal diet of rice straw at three different inclusion rates (0%, 10%, 20%, and 30%). The effects of *Morama* bean cake or sunflower seed cake on DM, CP, EE, ADF, ADL, cellulose, and non-fiber carbohydrates were significantly different after mixing with rice straw ($P < 0.05$). The amount of RMBC or WSSC added to rice straw increased its protein content and improved nutrient digestibility, ammonia concentration, and total volatile fatty acid formation ($P < 0.0001$).

The inclusion of 30% roasted *Morama* bean cake (RMBC) in the diet resulted in a reduction of various degradability metrics. Specifically, it decreased *in vitro* dry matter degradability (*ivADMdeg*) by 12.2%, *in vitro* true dry matter degradability (*ivTDMdeg*) by 5.4%, and *in vitro* organic matter degradability (*ivOMdeg*) by 4.6%. Therefore, it was concluded that RMBC would benefit ruminants at different incorporation rates, except for 30%, where the possible increased

levels of condensed tannins, polyunsaturated fatty acids, phytic acid, glucosinolates, and saponins in the oilseed cake could affect rumen microbial activity and overall feed degradability.

The studies underscore the potential of optimally processed *Morama* bean cake to enhance nutrient utilization and animal performance in ruminants. They also highlight the necessity of selecting the appropriate processing method and inclusion rate to maximize benefits while avoiding negative impacts from anti-nutritional constituents.

Keywords: Roasted *Morama* bean cake, whole *Morama* bean cake, *Morama* bean cake decorticated, whole sunflower seedcake

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Chapter 1

1.0 General Introduction

1.1 Background

Domestic ruminant production in sub-Saharan Africa has low productivity (Adesogan and Adeneye, 2002). The challenges faced by the livestock industry in this region include shortages and an unstable supply of quality feed (Makkar, 2000). A typical example is Botswana, where pasture clipping is carried out all year round, often showing low forage crude protein of 10% during summer (APRU, 1989), which drops to 6% in winter (Mthetho *et al.*, 2015). Although there are some shrubs and occasional hay that ruminants can feed on, they tend to contain antinutritional factors and high fiber content (Houston *et al.*, 1986). Previously, these were supplemented with animal protein or animal by-products from the Botswana Meat Commission or elsewhere on the market. Feeding animal protein or animal by-products in the form of carcass meal and blood meal has since been linked to mad cow disease (WHO, 2008). Antibacterial feed additives have also been banned for health reasons (Kilic *et al.*, 2011). This has created a gap in the availability of protein resources that small livestock farmers can access.

To allow effective microbial degradation of the forage, certain feed supplements (sunflower cake, alfalfa, soy, and *Dolichos lablab*) must be added. With the exception of *Dolichos lablab*, these feed supplements are exotic and sparsely grown in Botswana. Their importation incurs prohibitive costs for small-scale livestock farmers. However, in Botswana, there is a pool of native or indigenous plants (fruits/seeds/nuts) that have not been exploited by farmers but have the potential to provide essential nutrients for ruminant diets. There is evidence that some of them produce fruits

or nuts that are highly nutritious (Makhabu, *unpublished*; Motlhanka *et al.*, 2008). These include *Morula* (*Sclerocarya birrea*), African star chestnut (*Sterculia africana*), baobab (*Adansonia digitata*), and *Morama* (*Tylosema esculentum*). However, there is ample literature on the possible use of these plants in human diets (Mpotokwane *et al.*, 2013; Amonsou *et al.*, 2010; Museler and Schonfeldt, 2006), but less or none for ruminant diets.

The *Morama* bean (*Tylosema esculentum* Burchell A. Schreiber) is a wild legume adapted to the semi-arid conditions of southern Africa. The mature seeds of the *Morama* bean are encapsulated in hard, woody seed coats that are reddish to brownish-black in color (Van der Maesen, 2006). The seeds or beans are an important component of the diet among the nomadic “hunter-gatherers” in remote settlements, where conventional crop cultivation is hardly ever carried out (National Research Council, 1979).

Mature *Morama* seeds are known to store large amounts of lipids and proteins in the storage cotyledons, both well over 30% (Holse *et al.*, 2010), which is comparable to soybean (*Glycine max*) and peanut (*Arachis hypogaea*). The composition of fatty acids in *Morama* oil, as well as the content of phytosterols and vitamin E, were examined (Mitei *et al.*, 2009) and found to be abundant. Important essential amino acids such as tyrosine, arginine, leucine, and lysine have also been shown to be sufficient in *Morama* beans (Bower *et al.*, 1988). *Morama* beans have a fiber content that varies between 19% and 27%, a high content of lignans, but no cyanogenic glycosides or strong allergens found mainly in peanuts and lupins (Holse *et al.*, 2010).

In this study, *Morama* was chosen as a candidate for evaluating the nutritional properties of the nuts/beans in contrast to sunflower seed cake, a typical feed supplement for ruminants in southern

Africa. The reasons for this lie in the fact that the plant is abundant in the Kalahari Desert of Botswana, Namibia, and South Africa, and local communities use it sparingly. Therefore, this study aimed to expand the value chain of the plant. Sunflower seed cake has become so expensive that only a few farmers can afford it. Extensive research has been conducted on the nutritional properties of *Morama* beans in both poultry (Manyeula *et al.*, 2013) and human diets (Mpotokwane *et al.*, 2013), but there is no evidence to suggest that they have ever been used for ruminants. In order to determine the digestibility of the novel feed, it must be used directly by an animal (*in vivo* and/or *in situ*) or through laboratory-based methods (*in vitro*) that mimic the rumen environment.

1.2 Problem Statement

Digestibility of feed or diets can be achieved through various techniques, including the direct use of an animal (*in vivo* or *in situ*) and laboratory-oriented processes (*in vitro*) that mimic a rumen environment without directly using the animal. The degradability of feed ingredients is crucial for evaluating their use (Lei *et al.*, 2018). However, there are limited data on the *in vitro* or *in situ* degradation of nutrients from *Morama* bean cakes (MBCD, RMBC, and WMBC). The chemical composition of bean cakes varies slightly, possibly due to different processing methods.

While feed ingredients such as sunflower seed cake, soymeal, and fish meal are readily available in many stores, their prices are unaffordable for small farmers. Consequently, there is an urgent need for nutritional concepts that improve the production of more cost-effective feed formulations or nutritional supplements from native plant resources. Native plants, plant products, or by-products can provide ruminants with high-quality protein and enhance animal productivity (Beauchemin and McGinn, 2006).

1.3 Justification of the Study

Research has demonstrated that *Tylosema esculentum* (*Morama*) nuts is rich in protein (Amonsou *et al.*, 2010; Museler and Schönfeldt, 2006). However, tests of its functionality in feed systems have only been conducted on poultry (Manyeula *et al.*, 2013) and humans (Mpotokwane *et al.*, 2013). Knowledge regarding the use of its proteins and fatty acids in ruminants remains unknown. Given that energy is a limiting nutrient in grazing ruminants, lipids from *Morama* beans or nuts could potentially provide an energy advantage over carbohydrates (Belitz *et al.*, 2004). Utilizing *Morama* bean cake as a protein feed supplement could significantly reduce costs, particularly for small farmers. To date, there is no evidence in the literature that *Morama* bean cake has been used as a diet/feed supplement for ruminants in Botswana or elsewhere in the world.

This study is anticipated to have a significant positive impact on ruminant nutrition, particularly in rural areas where high-quality feed supplements are not easily accessible. *Morama* legumes are abundant in regions with marginal, low crude protein areas and limited vegetation resources, such as Kweneng West, Ngwaketsi West, Sandveld areas (Western Serowe), Western Maun, Gantsi, and Western Kgatleng District. Coincidentally, these areas also have a high population of ruminant animals, which often face nutritional challenges due to their economic importance in Botswana. Consequently, farmers in these regions will greatly benefit from the research findings provided in this study.

1.4 The aim of the study

Evaluate and compare the nutritional value of various processed *Morama* bean cakes (*Tylosema esculentum*) to that of sunflower seed cakes (*Helianthus annuus*).

1.4.1 Specific objectives:

- Determine the chemical and mineral composition and evaluate the dry matter (DM) and crude protein (CP) degradation properties of *Morama* bean cake compared to similar degradability parameters of sunflower seed cake using *in vitro* Daisy^{II} technique.
- Evaluate the dry matter (DM) and crude protein (CP) degradation properties of *Morama* bean cake compared to sunflower seed cake using *in situ* rumen technique, and compare the two methods (*in vitro* and *in situ*).
- Determine the *in vitro* degradability and digestibility of rice straw supplemented with roasted *Morama* bean cake or whole sunflower seed cake at varying inclusion rates, and compare the results.

1.5 Hypotheses

- *Morama* bean cake will exhibit comparable or superior chemical and mineral composition, as well as dry matter (DM) and crude protein (CP) degradation properties, compared to sunflower seed cake when evaluated using the *in vitro* Daisy^{II} technique.
- *Morama* bean cake will exhibit comparable or superior dry matter (DM) and crude protein (CP) degradation properties compared to sunflower seed cake when evaluated using the *in situ* rumen technique. Additionally, the *in vitro* method will be identified as equally effective in evaluating the nutritional value of ruminant feeds.
- Rice straw supplemented with roasted *Morama* bean cake will exhibit comparable or superior *in vitro* degradability and digestibility compared to rice straw supplemented with whole sunflower seed cake at varying inclusion rates.

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Chapter 2

2.0 Literature review

2.1 Introduction

Botswana is a sparsely populated country bordered by the Republic of South Africa to the south, and Zambia, Namibia, and Zimbabwe to the north, west, and east respectively. The country's only sea connections are via Namibia or South Africa. The average climate of Botswana is semi-arid, with seasonally irregular rainfall ranging from 250 mm in the southwest to 650 mm in the north (Batisani and Yarnal, 2010). The summer months (October to December) are hot, with typical daytime temperatures of 30°C (Botswana Meteorology, 2013). Winter temperatures range from mild to bitterly cold, especially in the evenings (Meteorology, 2013).

Approximately 80% of smallholder farmers in Botswana rely on livestock as a source of income (Sigwele and Orlowski, 2015; Statistics Botswana, 2020). Agriculture contributes 2.24% to the country's economy (World Bank, 2006), with sales of animal products and by-products accounting for 1.37% (Engelen *et al.*, 2014). Furthermore, the dominance of this sector in the rural economy accounts for the majority of nutrition, income, employment, and investment prospects (Statistics Botswana, 2020). Cattle production is the only source of foreign exchange in the livestock sector. This is evident from the fact that the government provides resources (vaccinations against diseases and traceability services) to the beef subsector at the expense of other agricultural production (Bahta and Malope, 2014; Temoso *et al.*, 2015). Despite the importance of livestock farming (cattle), there are declines in productivity. These problems are due to the inadequate forage productivity of the rangeland on which all grazing livestock production depends (Aganga and

Nsinamwa, 1997; Madibela *et al.*, 2000). According to Mosimanyana and Kilflewahid (1985), cited in Mthetho *et al.* (2015), the country's rangeland feed resources have high crude fiber content, low protein content, poor dry matter digestibility, and calcium-phosphorus imbalance. This lack of essential nutrients and minerals, along with the high content of fibre and minerals, negatively impacts livestock productivity.

2.2 Livestock performance

Livestock growth in Botswana has been declining since the late 1990s. As in other tropical or subtropical countries, ruminants in Botswana rely exclusively on pastures to meet their daily nutritional requirements (Madibela *et al.*, 2018). Despite their nutritional diversity (various plants, shrubs, and herbs), pastures remain the foundation of livestock nutrition for maintenance, development, and reproduction. Nutrient fluctuations in rangelands are caused by irregular rainfall, inadequate soil nutrition, and the type of forage available. This problem is exacerbated by deficiencies in certain minerals, particularly during the extended dry season (Nigele *et al.*, 2010). These issues are now further aggravated by climate change. During the dry season, most feedstuffs have a low crude protein (CP) content (5% to 6%) (Sowande and Sobola, 2008). Forages containing less than 6% CP do not support microbial protein synthesis in the rumen (Ørskov and Ryle, 1990). As a result, the levels of regenerated endogenous urea are low (Van Soest, 1994), leading to impaired feed intake and digestibility. To facilitate the recycling of endogenous urea, the minimum CP requirement in the diet of ruminants should be 7% (Getahun *et al.*, 2019).

To enhance performance, the nutrition of low-quality feed used as a basal diet can be improved through supplementation or processing (Aregheore and Rokomatu, 2004). Rumen microorganisms

produce amino acids by utilizing available nitrogen (N) sources such as ammonia and peptides. However, the synthesis of microbial protein in the rumen depends on the synergy between carbohydrates and nitrogen (Ørskov and Ryle, 1990). A lack of degradable intake protein (DIP) limits the use of inferior feed (Lintzenich *et al.*, 1995). An energy-DIP-synchronized supplementary feed improves feed and animal productivity (Lintzenich *et al.*, 1995). Therefore, it is necessary to understand protein requirements to avoid under- and/or over-supply.

2.3 Protein requirement of ruminants

The maintenance protein requirements of ruminants are fulfilled through the synthesis of microbial protein from both degradable and non-degradable dietary protein sources in the rumen. Soluble crude protein (CP) comprises a mixture of non-protein nitrogen (NPN) and true protein (TP) fractions (Van Soest, 1994). Both NPN and TP are readily degraded by rumen bacteria, whereas insoluble protein fractions vary from poorly degradable to highly non-degradable protein (rumen escape). Insoluble protein that escapes breakdown in the rumen is digested in the small intestine. However, not all insoluble protein fractions are digested in the small intestine (Babayemi *et al.*, 2014). The digestibility of microbial protein determines the availability of metabolizable protein expected to be absorbed in the small intestine (NRC, 2001; Van Soest, 1994). The utilization of metabolizable protein for production purposes depends on microbial protein production in the rumen, as well as the amount and digestibility of the non-degradable protein source available in feed ingredients (Gao *et al.*, 2015). Therefore, understanding the chemistry of different protein fractions in the rumen is essential.

Protein quality is crucial for providing nutritional benefits to animals (Hoffman and Falvo, 2004). Quality refers to the properties of the protein source in terms of its ability to achieve the intended

digestibility functions (Millward *et al.*, 2008). When assessing protein quality, consideration is given to a ration's ability to provide substrates needed for nitrogen synthesis and other biogenesis pathways, meaning an appropriate source of amino acids and nitrogen is included (Millward *et al.*, 2008). Evaluating protein quality involves assessing amino acid composition, bioavailability of amino acids, and digestibility (utilization of proteins), which are essential in predicting the metabolic function of the proteins in the animal (FAO, 1990). Various measurements and techniques are used to assess protein quality, which are explained in more detail in the following paragraph.

2.3.1 Characterization of Protein Fractions

According to the Cornell Net Carbohydrate and Protein System (CNCPS), crude protein (CP) fractions are divided into three levels (Chalupa *et al.*, 1991). These levels are classified as A, B, and C fractions. Fraction A represents non-protein nitrogen (NPN), fraction B represents true protein, and fraction C represents unavailable true protein. Fraction B is further divided into B1, B2, and B3 subgroups, which illustrate the different rates at which CP is broken down in the rumen. Fractions A and B1 are soluble in borate phosphate buffer and are therefore easily degradable in the rumen. The B2 fraction and some components escape degradation in the rumen and are digested in the lower intestine (Sniffen *et al.*, 1992). The degradation rates for fraction B3 are significantly slower than for fractions B1 and B2. This portion (B3) is inextricably linked to the cell wall and is not broken down in the rumen (Mondal *et al.*, 2008). The final component, fraction C, is known as acid-insoluble protein (ADIP) and is resistant to microbial and mammalian enzymes (Van Soest, 1994).

Heat coagulation can reduce the solubility of crude protein because amino groups and carbonyls form lignin-like matrices (Van Soest and Mason, 1991). This process can result in protein becoming partially indigestible, as shown in compartments B2 and B3, depending on the thermal pressure applied (Van Soest, 1994). Protein with a B2 degradation rate is suitable for rumen microorganisms that require a certain amount of energy for their degradation function. Albumin or globulin fractions of dietary proteins are more soluble than prolamin or glutelin fractions (Van Soest, 1994), contributing to easily degradable protein.

2.3.2 Dietary protein and fermentation process in rumen

Rumen degradable protein (RDP) is influenced by food intake (Saeed, 2008). Oilseed press-cakes exhibit CP degradation coefficients ranging from high to moderate (Van Soest, 1994). Due to the prolamin-zein effect, maize seeds, for example, are slowly broken down in the rumen (Van Soest, 1994). Prolamins tend to combine with water to form a waterproof and airtight matrix, that prevents starch granules from breaking down until fermentation (Momany *et al.*, 2006), so prolamins are not easily broken down. The type of bonds also influences the degradability of food proteins. Disulfide bonds within and between protein chains can influence CP degradability in the rumen, whereas connecting peptides can be responsible for differential CP degradability (Schwinger and Bates, 1996). Yang and Russell (1992) reported that feed containing lysine-proline degrades five times slower than feed containing lysine-alanine. Beckers *et al.* (1995) reported that proline-methionine in the diet of lambs was 250% slower acting. For effective protein utilization in the diet of ruminants, an appropriate ratio of roughage to protein concentrate is essential (Beckers *et al.*, 1995). Intake, specific gravity and particle size are factors that can influence both the interactions between rumen degradable protein (RDP) and non-degradable dietary protein (UDP) (Kamalak *et al.*, 2005). Rumen degradable protein (RDP) thus improves feed intake and

nutrient utilization (Putri *et al.*, 2021) of such low-quality feed. Supplementation with conventional protein optimizes digestible organic matter and duodenal CP flow. Khampa *et al.* (2003) reported that lambs supplemented with oilseed cake achieved a quadratic increase in body weight (BW) due to increased organic matter intake (OMI). Rumen degradable protein provides the rumen microbes with nutrients ($\text{NH}_3\text{-N}$), amino acids and peptides for survival and functionality (Putri *et al.*, 2021). Animal performance can be affected if nutrients are not supplied to the rumen microbial population (Khampa *et al.*, 2003). Cooper *et al.* (2002) reported that lambs fed a diet high in rumen degradable nitrogen (RDN) had a quadratic increase in feed conversion ratio (FCR) and average daily gain (ADG). Non-lactating dairy cows fed a low RDN diet had low feed intake and apparent digestibility (Braud, 2005). These above-mentioned studies thus demonstrate the importance of “feeding” rumen microbes with rumen-degradable protein.

2.3.3 Rumen undegradable protein (UDP)

Nondegradable dietary protein (UDP), also known as rumen bypass protein (BP-CP), is formed when dietary protein escapes degradation in the post-ruminant tract (Şehu *et al.*, 2010). The reason for providing UDP is to meet the metabolizable CP needs of ruminants (Gao *et al.*, 2015). A study by Yörk *et al.* (2006) has shown that UDP supports the lower tract of growing calves. The deamination resulting from UDP digestion help maintain a stable rumen environment and provides the ruminant with a consistent source of biodegradable nitrogen (Atkinson *et al.*, 2006). Increased UDP in ruminant diets improves N efficiency and amino acid delivery to the small intestine (Chiou *et al.*, 1995). Rusche *et al.* (1993) reported that calves fed diets fortified with rumen non-degradable protein (UDP) gained more weight. Habib *et al.* (2001) observed significant daily gains in grazing sheep supplemented with a high UDP diet. Therefore, feeds containing a mixture of

non-degradable and/or degradable protein improve the metabolizable protein of ruminants (Habib *et al.*, 2001) by increasing the transfer of organic matter to the lower tract.

2.3.4 Nitrogen and energy maximization in diets

Ruminants obtain amino acids from rumen fermentation contents (AFRC, 1992) and energy from volatile fatty acids (VFAs) provided by carbohydrate fermentation (Dewhurst *et al.*, 1986). Nutritional synchrony is a property that enables the use of RDP, which promotes microbial growth and efficiency (Hackmann and Firkins, 2015). Therefore, a balanced protein and energy supply in the diet is crucial for the growth rate and efficiency of microbial microorganisms (Sniffen and Robinson, 1987). Rumen microorganisms require a certain amount of nitrogen (60–80%) for protein synthesis, and any excess is converted into ammonia and released into the bloodstream and excreted by ruminants as urea (Miller *et al.*, 2001; Putri *et al.*, 2021). However, nitrogen recycling has limitations because it is time-dependent and relies on the balance between energy and nitrogen release (Bøsting *et al.*, 2003). Ruminants can effectively recycle Urea-N in the rumen. When the host animal is supplied with nutrients, N recycling is crucial for microbial nutrient supply (Marini and van Amburgh, 2003). Nitrogen recycling has limitations because it is time dependent and depends on the extent of imbalance between energy and N release (Bøsting *et al.*, 2003). The nitrogen recycling process is unable to temporarily buffer excess nitrogen (Valkeners *et al.*, 2006). The input of degradable energy instead of nitrogen inhibits microbial development and slows the digestive process in the rumen (Zain *et al.*, 2024). Proper synchronization of protein and energy results in a more effective fermentable diet for ruminants (Gleghorn, 2003). Herrera-Saldana *et al.* (1990) reported significant rumen efflux of microbial protein (MP) after sufficient harmonization of carbohydrate and protein intake. The authors also stated that the nutrients from such a strategy

increased milk production in dairy cows. Similarly, Matras *et al.* (1991) reported that improved performance in starch-fed lambs resulted in high retention of nitrogen sources.

Protein and energy synchronization has been extensively studied in both *in vitro* and *in vivo* studies (Aldrich *et al.*, 1983; McCarthy *et al.*, 1989; Stokes *et al.*, 1991; Arias *et al.*, 2020). The conclusion was that insufficient degradable protein was responsible for limiting rumen fermentation. There is a connection between ammonia (NH₃-N) intake and the fermentation process in the rumen (Hristov and Jouny, 2005); it has efficient microbial catalytic activity, and the ammonia content in the rumen should be in the range of 3 to 5 mg/100 ml (Satter and Slyter, 1974). Studies using different protein and energy sources showed that starch tends to influence the response to protein supply and thus alter protein utilization (Ipharraguerre *et al.*, 2005).

2.3.5 Effect of dietary protein on animal gain

Growing animals require more protein to ensure healthy tissue growth. Diets low in crude protein (CP) have deleterious effects on animals (NRC, 2001). Meta-analyses showed that the inclusion of different amounts of dietary protein in the diet of ruminants resulted in weight gains of about 0.38 g CP (ARC, 1980), 0.28 g CP (NRC, 1981), and 0.36 g CP (NRC, 1985). Titi *et al.* (2000) conducted a similar study on Awassi lambs and black billy goats with CP values of 12%, 14%, 16%, and 18%. Results showed that growth rates varied only between inclusions of 12% and 14% CP values, but there were no significant differences in weight gain in lambs fed diets with CP values of 16% and 18%. The CP incorporation rate of 16% resulted in the greatest weight gain of the animals. Insufficient calories caused the lambs fed 18% CP to have a reduced response and thus similar weight gain to the previous diet (Titi *et al.*, 2000). This shows that for optimal metabolism, high CP in the diet should be accompanied by high energy levels. Another problem

could be that the animals were at the limit of their protein requirements, which prevented increased protein accumulation. Small ruminants (sheep or goats) fed 0.38 g CP per day gained one gram per day (ARC, 1980) and 0.36 g CP per day (NRC, 1985), respectively, with no significant difference in growth. Davenport *et al.* (1995) showed a significant flux of nitrogen and microbial protein (MP) from the abomasum when the CP in lamb meal was increased from 14% to 16%, resulting in an increase in animal weight. When the crude protein content exceeded 16%, there was no significant change in average daily gain (ADG) due to insufficient energy supply, and the protein was converted into energy, which affected the degree of CP accretion (Davenport *et al.*, 1995). In summary, the above research shows that farmers should not feed protein in excess of requirements as it is a waste of resources, is costly, and can pollute the environment by excreting excess nitrogen. In particular, sufficient and available energy should always be provided.

2.3.6 Energy requirements of ruminants

Animal energy requirements depend on milk production, body condition, growth rate and type of activity due to weather conditions (Babayemi *et al.*, 2014). Lush grass or cut grass mixed with legume oilseed cake could provide energy to lactating or growing animals. For example, adding oil cake to a low-energy diet promotes animal weight gain and milk production by allowing more protein to be stored (Kubkomawa *et al.*, 2015). Chaudhary *et al.* (2022) demonstrated the importance of energy in animal nutrition by including low energy fiber in their study. Due to inadequate energy supply, animal treatments resulted in a significant reduction in energy metabolism and overall feed digestion (Chaudhary *et al.*, 2022) therefore additional energy would be required. Therefore, seed oil cake, though are provided as protein source also supply the necessary energy from its lipid content (Kubkomawa *et al.*, 2015).

2.4 Mineral requirements of ruminants

Minerals play important roles in cellular respiration, nervous system development, protein synthesis, metabolism, reproduction (McDowell and Arthington, 2005), and immune function. The availability and concentration of minerals in feed are influenced by the interaction between soils, plants, dry matter, and plant maturity (Khan *et al.*, 2005). The majority of minerals are obtained through forage, concentrated feed, and mineral supplements. Minerals, particularly sulfur and phosphorus, are required for protein synthesis by rumen microorganisms (Sniffen and Robinson, 1987), as well as for energy and bone metabolism.

Mineral concentrations in the subtropics and tropics vary depending on the ecotone of the soil cluster (Njidda and Olatunji, 2012). The Sandveldt ecotone in southern Africa (SADC region) is phosphorus deficient compared to the Hardveldt ecotone, which has a significant amount of mineral deposits in the soil, particularly during the rainy season (APRU, 1989). The lack of phosphorus in winter (dry period) is exacerbated by the lignification of the forage and binding by phytic acid, making it no longer available. Therefore, this mineral must be supplemented as a lick or provided in a concentrate (APRU, 1989).

Ruminant nutritionists need to assess the availability of macro- and micro-elements in the correct proportions to inform farmers about mineral imbalances in animal nutrition. The supply of lipids is often neglected, especially in ruminants, but essential fatty acids are crucial for producing healthy animal foods for human consumption.

2.5 Effects of Fatty acids intake on animal performance

Essential fatty acids in ruminant diets improve maintenance, development, and reproductive efficiency (Santos *et al.*, 2008). Due to microbial biohydrogenation, ruminant muscles naturally contain fewer essential fatty acids than those of non-ruminants (Juchem *et al.*, 2010). Ferguson *et al.* (1989) found that feeding fatty acids to dairy cows increased conception rates by 2.2%. Fatty acids in dairy diets improve embryo survival in pregnant cows (Mattos *et al.*, 2000). In addition to their importance, fatty acids can promote the fluidity of follicles (Zachut *et al.*, 2008), oocytes (Zeron *et al.*, 2002), and embryonic development (Thangavelu *et al.*, 2007).

Unsaturated fatty acids in the diet can alter ovarian steroidogenesis and thus follicle concentration (Moallem *et al.*, 1999). According to Robinson *et al.* (2002), dairy cows fed a diet high in linolenic acid show an increase in steroid concentrations compared to the control group (the diet did not contain any fatty acid). Moallem *et al.* (1999) also supplemented cows with unsaturated fatty acids from oilseed cakes and found similar results. The scientists found that cows fed saturated fat had increased follicular steroid concentrations. Staples *et al.* (1998) reported that unsaturated fatty acids promote reproductive success by altering progesterone metabolism.

Galbreath *et al.* (2008) suggested that oilseed cake supplementation could reduce cases of low progesterone or corpus luteum, allowing cows to remain pregnant. Bilby *et al.* (2006) reported that progesterone concentrations in ruminants supplemented with oil cake were due to the presence of long-chain fatty acids rather than unsaturated fatty acids (UFA). These authors also discovered an increase in immunoglobulin (IgG) in calves (from 23.3% to 27.9%) when their dams were fed an oil cake diet. The calves gained more weight each day when suckling dams on the diet compared to control calves.

According to Jose *et al.* (2013), first calvers fed a diet high in oilcake (linoleic acid) produced more milk than those fed a milk replacer low in linoleic acid. Some researchers (Staples *et al.*, 1998; Moallem *et al.*, 2010; Jose *et al.*, 2013) hypothesized that supplementing the diet of ruminants with unsaturated fatty acids, especially during prenatal preparation, results increased animal productivity, such as milk production. Livestock production in southern Africa is challenged by the lack of quality feed ingredients for animal nutrition, and there is a constant search for new and sometimes unconventional feedstuffs.

Therefore, this review compares a potential new protein source for ruminants, *Tylosema esculentum* (*Morama*) bean cake, with a long-established traditional dietary supplement, sunflower seed cake (Richardson and Anderson, 1981; Vasudha and Sarla, 2021), which is a byproduct of oil production from black oil seeds. Sunflower seed cake is described using meta-analysis examples from various research studies. The proposed use of the novel feed supplement (*Morama* bean cake) aims to serve as an alternative protein source for ruminants.

2.6 Sunflower (*Helianthus annuus*) as an ingredient in ruminant feed

Sunflower seed cake has long been used as a protein source in ruminant diets (Richardson and Anderson, 1981). However, it provides low-quality crude protein for ruminants (Ngongoni *et al.*, 2009). Heat treatment or roasting of seeds after solvent extraction could increase the non-degradable protein content, resulting in high metabolizable energy for ruminants (NRC, 1996). According to McGinn *et al.* (2004), 5% sunflower oil in a forage-based ruminant diet reduced methane emissions by 17%. Sunflower energy, like crude protein, has less value than certain oilseed cakes (NRC, 1996). The energy levels differ depending on the fiber and residual oil concentration (Table 2.1). The oil extraction technique affects energy storage in the cake, with a

higher proportion of husks in the mill reducing the energy value and feed cake volume (NRC, 1996). Similarly, mechanical oil extraction leaves a higher oil residue in the cake, providing high energy content in the diet of ruminants (Jordan *et al.*, 1997).

Table 2.1: Nutrient content of solvent extracted sunflower cake based on number of hulls retained

Item	Unshelled seeds	Partial dehulled	Dehulled
DM ¹ , %	90.00	90.00	90.00
CP ²	28.00	34.00	41.00
EE ²	1.50	0.80	0.50
CF ²	24.00	21.00	14.00
Ash ²	6.20	5.90	5.90
Ca ²	0.36	0.35	0.34
P ²	0.97	0.95	1.30
K ²	1.07	1.07	1.07
Mg ²	0.80	0.79	0.79

Source: Hesley (Ed.) National Sunflower Association, 1994 ¹DM – dry matter as fed

²CP – crude protein on dry matter basis; ²EE – ether extract on dry matter basis; ²CF – crude fiber on dry matter basis; ²Ash – on dry matter basis; ²Ca – calcium on dry matter basis; ²P – phosphorus on dry matter basis; ²K – potassium on dry matter basis; ²Mg – on dry matter basis

2.6.1 Meta-analysis of sunflower seedcake

Some authors (Anderson and Lardy, 2012; Ngongoni *et al.*, 2015) found that sunflower meal is highly degradable in the rumen compared to other existing feed additives such as soybean, rapeseed, and cottonseed meal. However, soybean, rapeseed, and cottonseed meals have higher energy values than sunflower meal (NAS, 2016). The low energy value of sunflower meal is related to its fibre and oil content, which also minimizes its bulk density (Hesley, 1994).

Landblom *et al.* (1987) compared sunflower meal (percentage crude protein) to soybean and barley distillers' grain in developing heifer diets and found them to be 1.09%, 1.12%, and 1.12%, respectively. In terms of feed efficiency, barley distillers' grain was 6.49 g, while soybean and sunflower meal were 6.76 g and 6.90 g, respectively.

Richardson *et al.* (1981) conducted a feeding trial in which they replaced sunflower meal with cottonseed meal in the rearing and final feeding of steers at 0%, 5.5%, 11%, and 22% dry matter (of total feed intake). Workers reported digesting feed containing isonitrogenous and fiber content with up to 11% sunflower oil. Additionally, the researchers pointed out that sunflower oil not only provides energy to ruminants but can also reduce methane production by 22%.

However, sunflower by-products are prohibitively expensive for small livestock farmers, particularly in rural areas of the SADC region. This is because sunflower grain seeds are imported, and the high costs have recently been exacerbated by the war in Ukraine. Consequently, a cost-effective alternative to traditional protein-rich sources must be found.

Morama bean cake (*Tylosema esculentum*) is hypothesized as a potential option for inclusion in ruminant diets. Evidence of its potential in the diet of monogastric animals has been documented (Bieseke and Murray, 1983; Armateifio and Mholo, 1998; Museler, 2005; Manyeula *et al.*, 2013). Additionally, apart from the expenses associated with sunflower cake for farmers, a study by Alves *et al.* (2016) has highlighted the shortfalls in sunflower cake or sunflower seedcake, as presented in Table 2.2.

The intake of nutrients, including dry matter, organic matter, crude protein, ether extract, neutral detergent fiber, and metabolizable energy, varied significantly among the different protein sources such as, soybean meal (SBM) castor bean cake (CB), sunflower cake (SC), and sunflower seedcake (SS). Specifically, SBM and CB generally had higher nutrient intakes compared to SC and SS.

In conclusion, researchers have demonstrated that the choice of protein source significantly impacts both the intake and digestibility of nutrients in lambs. Soybean meal (SBM) and cottonseed meal (CB) appear to be superior protein sources compared to sunflower cake (SC) and sunflower seedcake (SS) in terms of both nutrient intake and digestibility. This finding clearly indicates that the replacement of sunflower seedcake as a profitable protein source is highly desirable.

Table 2.2: Intake and digestibility of nutrients of lambs fed sunflower mill, seed cake and other protein sources

Item (Intake kg d ⁻¹)	Protein source				SEM	P-value
	SBM	CB	SC	SS		
Dry matter	0.989	0.964	0.857	0.789	0.002	***
Organic matter	0.919	0.889	0.791	0.733	0.001	***
Crude protein	0.146	0.14	0.125	0.108	0.001	***
Ether extract	0.04	0.05	0.046	0.069	0.0001	***
Neutral detergent fiber	0.409	0.447	0.391	0.352	0.003	***
Metabolizable energy (Mcal kg ⁻¹ DM)	2.59	2.63	2.15	1.666	0.022	***

<i>Digestibility (g/kg)</i>						
Dry matter	690	700	630	510	0.003	***
Organic matter	700	710	640	520	0.002	***
Crude protein	640	690	620	490	0.001	***
Neutral detergent fiber	510	610	490	350	0.002	***

Source: Alves *et al.* (2016)

SBM – soybean; CB – castor bean cake; SC – sunflower cake; SS – sunflower seed cake; SEM – standard error of the mean; significance *** P < 0.001

2.7 *Tylosema esculentum* (morama) plant

Tylosema esculentum, also known as *Morama* or *Marama* bean (Botswana and Namibia; Setswana), *Braaiboontjie*, *Maramaboontjie*, and *Elandsboontjie* (Republic of South Africa and Namibia; Afrikaans) (Van Wyk and Gericke: 2000) and gemsbok bean in English (Smith, 1966), is a wild, creeping angiosperm from the family Leguminosae (Fabaceae). *Tylosema esculentum* (Burch) Schreiber, *Tylosema fassoglensis* (Kotschy) Torre and Hillc, *Tylosema argenteum* (Chiov) Brenan, and *Tylosema humifusum* (Pichi - Serrn and Roti - Michael) Brenan are the four known species. *Tylosema esculentum* (*Morama*) occurs in southern Africa (South Africa, Namibia and

Botswana). The remaining three species occur in East and Central Africa (Ethiopia, Somalia, Kenya, Zambia, and the Democratic Republic of Congo). In Botswana, *Tylosema esculentum* (esculentum=edible) occurs in the northern districts of Kgalagadi, Gantsi, Ngwaketsi West, Western Central, Kweneng West, Ngamiland and Western Kgatleng (Ramolemana, 2003).

Tylosema esculentum (Burchell) A. Schreiber grows best on sandy-clay soils with traces of dolomite and limestone (Ramolemana, 2003; Amonsou *et al.*, 2011). Flowering begins in summer and continues until fall when pods form. The pods have an irregular oval and elongated morphology and are 1.3 to 2 cm high and 1.5 cm wide (Amonsou *et al.*, 2010). Each pod contains two seeds, the colour of which range from brownish-black to dark depending on the state of ripeness and the canopy of the higher branching tree branches. *Morama* is dormant in winter and obtains nutrients through underground tubers (Castro *et al.*, 2005). Although *Morama* vines and leaves are grazed, they are not the most popular livestock feed due to the bitter taste, perhaps due to presence of hydrolyzable tannin, terpenes, sesquiterpenes (Museler, 2005).

2.7.1 Nutritional Attributes of *Morama* beans

2.7.1.1 Proximate nutritional composition

Table 2.3 illustrates the variations in dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acidic detergent fiber (ADF), and other properties such as essential fatty acids, as observed in numerous research studies (Museler, 2005; Amonsou *et al.*, 2011). *Morama* beans have a crude protein content of 364.4 g/kg DM, an ether extract (EE) content of 368.2 g/kg, and a dry matter (DM) content of 938.5 g/kg (Museler, 2005; Jideani *et al.*, 2009; Amonsou *et al.*, 2011). The

nutritional quality of Morama seeds can vary based on soil type and pH levels (Ramolemana, 2013).

Table 2.3: Chemical composition (g/100 g) of *Morama* bean in comparison with that of soybean

Samples	Moisture	Protein	Fat	Crude fiber	Ash	Total carbohydrate
<i>T. esculentum</i> (SA)	7.0 ^b ±0.2	30.6 ^a ±0.2 (32.9)	32.9 ^c ±0.5 (35.4)	7.9±0.1 (8.5)	3.3 ^a ±0.1 (3.5)	18.3 ^a ±0.7 (12.7)
<i>T. esculentum</i> (BW)	5.1 ^a ±0.7	30.6 ^a ±0.3 (32.2)	36.2 ^d ±0.3 (38.1)	8.1±0.2 (8.5)	3.2 ^a ±0.7 (3.3)	16.8 ^a ±0.3 (12.8)
Soybean	7.9 ^b ±0.8	32.5 ^b ±0.2 (34.9)	14.9 ^a ±0.1 (16.2)	8.6±0.2 (9.3)	4.8 ^b ±0.7 (5.2)	31.3 ^b ±1.3 (26.3)

Source: Amonsou, 2010

SA: South Africa, BW: Botswana; Mean ± SD – mean values with different superscript letters in columns are significantly different (P<0.05); values in parentheses are mean values expressed on a dry matter basis; Total Carbohydrates by difference

2.7.1.2 Amino acid content of morama seed

The crude protein (CP) content of *Morama* seeds/beans accurately reflects the amount of amino acids contained in the seeds. The content of individual amino acids (AA) in *Morama* seeds ranges from 4 to 133 g/kg DM, with glutamic acid (31.7–133 g/kg DM) and lysine (5.6–41 g/kg DM) having the highest amounts among non-essential and essential amino acids, respectively (Table 2). Since *Morama* seeds are rich in essential amino acids, they can benefit both the rumen microorganisms and the host animal through the rumen bypass procedure (Reveneau *et al.*, 2008). Some of the amino acids present in *Morama* nuts are listed in Table 2.4.

Table 2.4: Amino acid composition (g/100g protein) of morama seed reported in some sources*

Amino acid (g/100g protein)	N‡	Mean	Range	SD
Arginine	4	2.66	1.46 - 4.5	1.62
Serine	4	2.98	0.98 - 7.00	3.48
Aspartic acid	4	4.70	2.20 - 9.50	4.16
Glutamic acid	4	6.55	3.08 - 13.30	5.85
Threonine	4	1.89	1.13 - 3.40	1.31
Glycine	4	4.13	1.26 - 9.70	4.83
Alanine	4	2.38	1.09 - 4.80	2.10
Tyrosine	4	4.80	3.36 - 7.70	2.50
Proline	4	3.52	1.22 - 8.00	3.88
Methionine	4	2.28	2.26 - 2.29	0.02
Valine	4	2.17	1.06 - 4.30	1.85
Phenylalanine	4	1.83	1.09 - 3.3	1.27
Isoleucine	4	1.87	1.06 - 3.30	1.25
Leucine	3	2.76	1.24 - 5.50	2.38
Histidine	2	1.08	0.60 - 1.90	0.72
Lysine	3	2.07	1.05 - 4.10	1.76
Tryptophan	2	0.68	0.67 - 0.70	0.02
Cystine	2	0.51	0.40 - 0.56	0.09

*Supporting literature: Múselér *et al.*, 2005, Jideani *et al.*, 2009; Amonsou *et al.*, 2010; Mosele *et al.*, 2012; ‡Number of supporting literature

2.7.1.3 Fatty acid content of *Morama* seeds

Morama seeds/beans have a crude fat content of 33.5–40.73 g/kg DM (Table 2.3). The ratios of unsaturated to saturated fatty acids are between 2.5:1.0 and 5.3:1.0 (Museler and Schonfeldt, 2006). Palmitic acid (12.8%) and stearic acid (7.9%) are the main saturated fatty acids (SFA) found in seeds/beans, while oleic acid (42.03%) and linoleic acid (32.2%) are the main unsaturated fatty acids (UFA) found in seeds/beans. According to Museler and Schonfeldt (2006), *Morama* seeds lack alpha-linolenic acid. Because mammals lack fatty acid daturases, both linoleic acid (LA) and

alpha-linolenic acid (ALA) are essential in the diet of ruminants (Zhu *et al.*, 2017). The fatty acids linoleic acid (LA) and alpha-linolenic acid (ALA) are required in the diet of ruminants because they cannot be synthesized by the animals and must be supplied through feed (Elliot *et al.*, 1989). Essential fatty acids are beneficial in the diet of ruminants because they slow the ammonia formation process and increase rumen bypass protein delivery to the hindgut (Losa, 2001). Here is the detailed information from Table 2.5 on the fatty acids found in *Morama* nuts:

Table 2.5: Fatty acid profile of Botswana morama bean compared to the Namibian morama bean

Fatty acid	Botswana bean ¹	Namibia bean ²	M ± SD ^{1,2}
Palmitic (C16:0)	13.62	13.98	13.80 ± 0.25
Palmitoleic acid (C16:1)	0.53	0.58	0.56 ± 0.04
Stearic acid (C18:0)	8.47	7.63	8.05 ± 0.59
Oleic acid (C18:1)	45.91	38.41	42.16 ± 5.30
Linoleic acid (C18:2)	27.32	34.89	31.11 ± 5.35
Linolenic acid (C18:3)	0.00	0.00	0.00 ± 0.00
Arachidic acid (C20:0)	2.43	2.56	2.50 ± 0.09
Arachidonic acid (C20:1)	0.41	0.39	0.40 ± 0.01
Behenic acid (C22:0)	1.31	1.56	1.44 ± 0.18

Source: Müsseler, 2005

2.7.1.4 Minerals

Morama nuts contain sufficient trace and major minerals, except for the calcium to potassium ratio (Müsseler, 2005). The mineral concentration varies depending on the soil type (Ramolemana, 2013). The most important minerals are present in the following amounts: 0.41 to 0.50 mg/g DM for phosphorus, 0.21 to 0.30 mg/g DM for calcium, 273 to 276 mg/kg DM for magnesium, 836 to 954 mg/kg DM for potassium, and 0.84 to 1.00 mg/g DM for sodium (Müsseler and Schönfeldt,

2006). Morama beans have a very low calcium to phosphorus ratio in both Botswana (0.21:0.41) and Namibia (0.30:0.50) (Table 2.6) and therefore require mineral supplementation for ruminants (Museler & Schönfeldt, 2006).

Table 2.6: Mineral composition of *Morama* bean (g/100 g)

Minerals	Botswana ¹	Namibia ²	M ± SD ^{1,2}
Phosphorus (mg/100 g)	0.41	0.50	0.46 ± 0.05
Calcium (mg/100 g)	0.21	0.30	0.26 ± 0.05
Magnesium (mg/100 g)	0.28	0.27	0.28 ± 0.01
Potassium (mg/100 g)	0.84	1.00	0.92 ± 0.10
Sodium (mg/100 g)	0.06	0.07	0.07 ± 0.01
Chloride (mg/100 g)	0.06	0.07	0.07 ± 0.01
Fluorine (mg/100 g)	0.001	0.001	0.00 ± 0.00
Iodine (mg/100 g)	0.002	0.001	0.00±0.00
Cobalt	0.016	0.0020	0.00±0.00
Iron	0.310	0.400	0.00±0.00
Zinc	0.600	0.640	0.00±0.00

Source: Müsseler, 2005

2.7.1.5 Quantification of seed protein

It has been shown that the amino acid content of seed proteins is significantly lower than that of leaf proteins (Van Soest, 1994). The seed protein concentration is often insoluble and inaccessible to grazing species (Van Soest, 1994). Prolamins, glutelins, albumins, and globulins are protein stores in *Morama* beans (Van Soest, 1994). Prolamins are highly soluble in alcohols but insoluble in water and salt solutions. Glutelins are only soluble in dilute alkali. Albumins are soluble in water but insoluble in alcohol, and globulins are soluble in salt solutions but insoluble in water and alcohol (Van Soest, 1994).

The high protein content of prolamins and glutelins in the Graminea family makes them hydrophobic and insoluble (Momany *et al.*, 2006), which may hinder degradation in the rumen. In contrast, dicotyledon seeds, particularly legume seeds, contain globulins and albumin proteins

(Van Soest, 1994). When these proteins are exposed to heat, they become denatured and water-insoluble (Conrad and Klopfenstein, 1988; Van Soest, 1994). The solubility of the seed proteins relevant to rumen utilization are albumin and prolamins, the latter being sufficiently utilized if the seeds have been previously fermented (Momany *et al.*, 2006). *Morama* seeds contain 23.3% albumin, 5% globulin, 7.7% glutelins and 15.5% prolamins (Museler and Schonfeldt, 2006). However, sunflower seed cake, a popular nutritional supplement for ruminants, contains 20% albumin (Kortt and Caldwell, 1990), 38% globulin (Youle and Huang, 1981), and trace amounts of glutelins and prolamins (Zilic *et al.*, 2010). The presence of prolamins in *Morama* beans is uncommon among legumes (Boutler, 1977), and this could make CP degradation in the rumen impossible unless prior fermentation or roasting occurs (Momany *et al.*, 2006). The feeding value of *Tylosema esculentum* cake can only be come apparent if it is analyzed before feeding to animals.

2.7.2 Techniques for measuring nutritive value of feeds.

Before feeding animals, laboratory analysis is required to determine the nutrient content of novel feeds. This can be done using wet chemistry, near-infrared spectroscopy (NIR), *in vitro*, and/or *in situ* methods. By using these procedures, the researcher would not expose the feed (safe for *in situ*, but not *per os*) to the animal that would eventually feed on it. The results could be partially applied to how the nutrients would be digested and assimilated by the subject (animal). According to Madibela *et al.* (2003), chemical analysis of new feed components is important, but for ruminants, it is not sufficient because it does not take into account what happens in the rumen.

In vivo studies are the most reliable method for assessing nutrient digestibility and utilization of feed because there is an actual response from the ruminant (Lopez, 2005). However, this form of

research is expensive and time-consuming and therefore unattractive for most people. Alternative methods include *in vitro* and *in sacco* methods that could be used to predict degradation kinetics or apparent digestibility of foods (Van Soest, 1994; Kitessa *et al.*, 1999). These approaches (*in vitro* and *in situ*) were examined in detail below.

2.7.2.1 *In vitro* feed assessment techniques

There are numerous feed evaluation methods available for use in ruminant feed. After the development and use of McDougall buffer (Weiss, 1994), the *in vitro* approach became even more important as it allowed feed to be incubated for longer periods of time. The buffer maintains the pH at about 6.7 and reproduces the rumen environment, which is excellent for the establishment of cellulolytic bacteria and fiber digestion (Russell *et al.*, 1979). Despite the differences that may occur between *in vitro* techniques, they share common analytical procedures for assessing post-incubation residues and/or degradation/digestion metabolites. For this current study, *in vitro* techniques using the Daisy^{II} incubator were used to both assess feed parameters and determine feed interactions during digestion; The *in situ* method was also used to measure the rate and/or extent to which feed parameters were degradable (Broderick and Cochran, 1999). Therefore, some of the methods/techniques used are reviewed to highlight their importance in providing basic information about the feed in question before it can be used/fed to ruminants.

2.7.2.2 Continuous culture

This technique tends to propagate rumen microbes at suboptimal growth rates, resulting in a constant (steady-state) microbial concentration number (Hoover *et al.*, 1976). By adding buffers and nutrients, a vibrant microbial concentration is maintained while fermentation products are

continuously removed to achieve a steady state that simulates the rumen environment. This method is used to measure feed parameters (dry matter, crude protein, neutral detergent fibre, etc.) and provides values comparable to *in vivo* data (Calsamiglia *et al.*, 2000). The continuous culture system (*in situ*) is normally used and has the advantage of modifying the rumen environment, thereby facilitating the assessment of microbial growth (Doreau and Ould-Bah, 1992). On the other hand, the *in vitro* technique is used to quantify fermentation products such as ammonia and volatile fatty acids (VFAs) produced during feed incubation. By quantifying volatile fatty acids, scientists can assess the nutritional value of each feed. VFAs have been postulated to provide half of the digestible energy nutrient intake of ruminants (France and Dijkstra, 2005). The most important VFAs in ruminant diets include acetic acid, propionic acid, and butyric acid. These VFAs provide ATP that animals require for their daily functions (Dijkstra, 1994).

The other use of the *in vitro* method is to measure crude protein degradation by determining ammonia concentration during feed incubation (Raab *et al.*, 1983). Protein breakdown in the rumen is the first phase of protein assimilation before exposure to the gastrointestinal juices in the abomasum. When ammonia is produced beyond microbial utilization, it is absorbed through the rumen wall, detoxified in the liver, and excreted from the animal body in the urine as urea (Pacheco and Waghorn, 2008). Notwithstanding the above, ammonia measurements can be influenced by the substrate used, the incubation time and the quality of the buffer added to the rumen fluid (Michalet-Doreau and Ould-Bah, 1992).

2.7.2.3 *In-situ* method of feed assessment

In this type of technique, non-degradable porous bags containing some food are inserted into a cannulated animal (cow, sheep, or goat) to measure degradability over a period of time (24, 48, 72) (Lopez, 2005). This method measures the ruminal degradability of feed or food components (Broderick and Cochran, 1999). The *in situ* technique is a perfect predictor of feed degradability and has been proven to provide much higher accuracy than the *in vitro* method (Gosselink *et al.*, 2004; Ørskov, 2000). The method enables an improved assessment of crude protein breakdown in the rumen and is considered a groundbreaking method for determining the protein requirements of ruminants (Broderick and Cochran, 1999). The design of the bags used in this method is such that the pore size allows the free flow of fluid and microbes between the bag and the rumen. However, the loss of undigested feed must be avoided at all costs (Vanzant *et al.*, 1998). Lopez (2005) recommends bags with pore sizes between 35 and 55 microns. Lining material placed in the bag could affect the technique as the bag should not be overfilled. This delays bacterial colonization and increases the lag time (Burke, 2004). The ideal ratio of lining material to bag surface should be 10 and 15 mg/cm² to facilitate microbial colonization (Hvelplund and Weisbjerg, 2000). The amount of feed in bag should be sufficient for a residue analysis (Nocek, 1988). The method has limitations because the cannulated animal may die or suffer some other mishap that may affect its use. Therefore, modeling is of utmost importance when using the method to predict future equations to avoid situations where cannulated animals are not available.

2.8 Conclusion

The lack of protein sources in the diet of sub-Saharan ruminants, particularly in Botswana, has necessitated research into more cost-effective animal feeds that contain sufficient proteins with a

significant fatty acid profile. The use of conventional protein sources of plant origin is limited by their seasonality and the associated high prices, such as sunflower seed cake. Furthermore, the use of unconventional protein sources is limited by the presence of secondary metabolites. However, these ingredients can be processed or treated to reduce the concentration of anti-nutritive factors, making the ingredients more suitable as potential protein sources for animal feed. The use of unconventional plant protein sources for possible incorporation into animal feed as a replacement and/or supplement to expensive conventional sources is inevitably necessary. Several studies have shown that unconventional protein sources (seed cakes, etc.) can be integrated into animal nutrition without negative effects on animal growth performance, milk composition, carcass characteristics and meat quality. Therefore, it is worth exploring the potential of *Morama* bean cake as an unconventional protein source in ruminant feed.

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Chapter 3

***IN VITRO* AND *IN-SITU* RUMINAL DRY MATTER AND CRUDE PROTEIN DEGRADABILITY OF THREE DIFFERENT *MORAMA* BEAN (*MORAMA* BEAN CAKE DECORTICATED, ROASTED *MORAMA* BEAN CAKE, AND WHOLE *MORAMA* BEAN CAKE) AND SUNFLOWER SEED CAKES**

ABSTRACT

The objective of this study was to determine the chemical and mineral composition of various processed *Morama* beans (decorticated *Morama* bean cake, whole *Morama* bean cake, and roasted *Morama* bean cake) and whole sunflower seed cake (WSSC). Additionally, the study also aimed to evaluate the feed samples dry matter (DM) and crude protein (CP) degradability using two different techniques: (i) *in vitro* Daisy^{II} and (ii) *in situ* methods.

The samples were first deoiled in an electric oil extractor, either with the seed coat intact or decorticated. Only one protein source (RMBC) was roasted in a domestic oven at 250°C for 20 minutes. Minerals were determined using an inductively coupled plasma-optical emission spectrometer (Perkin-Elmer, OPTIMA - 3300 RL). After oil extraction, samples were ground through a Wiley mill (1 mm sieve) using standard analytical procedures to determine chemical composition, minerals, and fatty acids. For *in vitro* and *in situ* incubations, samples were ground with the same equipment but using a 2 mm sieve.

In a Daisy^{II} device, four feeds (1.25 g DM) were placed in nylon bags in pooled rumen fluid from three cannulated Holstein-Friesian cows fed *Eragrostis rigidior* grass hay combined with alfalfa haylage. This was done in a complete factorial design (4 inocula x 4 substrates) for 2, 4, 6, 12, and 24 hours, repeated twice. To create the degradability profiles, the nonlinear (NLN) model of Ørskov and McDonald was used, fitting the DM and CP data into the exponential equation ($p = a + b (1 - e^{-ct})$), where (a) is the rapidly degradable fraction and (b) is the slowly degradable fraction.

For *in situ* rumen incubation, four samples (5 g DM) were replicated three times and randomly assigned to three cannulated Tswana steers in a completely randomized design (CRD) over two periods (4 feeds x 3 replicates x 3 steers) for 0, 2, 4, 6, 8, 16, 24, and 48 hours also repeated twice. The steers were fed two equal portions of the total mixed ration (TMR) twice daily. DM and CP degradability data were fitted to the equation ($Y = a + b (1 - e^{-ct})$), with (a) and (b) fractions defined as above. The results of the data on DM and CP degradation fractions and chemical constituents were used to create prediction equations assuming rumen flow rates of 0.02, 0.05, and 0.08/h for both techniques.

The chemical composition results showed differences between the four feed samples in ADL value ($P < 0.05$) and cellulose content ($P < 0.01$). WMBC and WSSC did not differ ($P > 0.05$) for CP, ADF, and NDF content, but both differed ($P < 0.05$) from RMBC and MBCD for the same components. WMBC and MBCD did not differ ($P > 0.05$) for DM, EE, and hemicellulose content, but both differed ($P < 0.05$) from RMBC and WSSC for the same components. WMBC and RMBC did not differ ($P > 0.05$) for CT, ash, and OM contents, but both differed ($P < 0.05$) from MBCD and WSSC for the same components. RMBC and WSSC did not differ ($P > 0.05$)

for NFC and ME content, but both differed ($P < 0.05$) from WMBC and MBCD for the same components.

The mineral results for calcium content in all feed samples were comparable (0.2% DM; $P > 0.05$), except for MBCD, which had a lower content (0.1% DM). For other major minerals such as P, K, and Mg, concentrations were 0.4%, 0.9%, and 0.1% for WMBC, MBCD, and RMBC, respectively. There was no significant difference ($P > 0.05$) in Na content between feed samples. *In vitro* MBCD treatment had a significant impact on the disappearance of DM and CP, effective dry matter degradability (EDMD), and effective crude protein degradability (ECPD) ($P < 0.05$) at 24 hours. *In situ* results showed that CP a , b , and k_d parameter values for different feed treatments were lower ($P < 0.05$) than the corresponding DM values. Specifically, RMBC had the highest DM disappearance, but roasting impaired its effective CP degradability.

When comparing the degradation kinetics, both methods (*in vitro* and *in situ*) appeared to be consistent, except for the effective degradation parameters in the *in vitro* technique. The discrepancies between *in vitro* and *in situ* degradation kinetics, including effective degradability (ED), were expressed using the degradation quotient (degQ), where $degQ =$

$$\frac{\text{in vitro}(a,b,u,k_d,ED) - \text{in situ}(a,b,u,k_d,ED)}{\text{in situ}(a,b,u,k_d,ED)}.$$

The process for predicting the kinetic degradation fractions of DM and CP was based on a statistical relationship between the kinetic degradation and the chemical components, sometimes resulting in equations with little biological significance. The DM a of MBCD and WMBC samples were best described by CT + ADF ($R^2 = 0.968$) and ADF ($R^2 = 0.565$) as dual and single predictors, respectively. The effective crude protein degradability (EDCP) of WSSC and RMBC had the

highest ($P < 0.05$) R^2 values of 0.739 and 0.999, respectively. Therefore, it was concluded that *Morama* bean cake had higher DM and CP availability to rumen microbes than sunflower seedcake. However, the potential of *Morama* bean cakes as a feed supplement for ruminants would need to be validated under *in vivo* conditions, as the secondary compounds contained in the bean cake could offset the positive influence of the nutritional value of *Morama* on animal performance. The extended *in situ* rumen incubation is expected to provide better reproducibility than the *in vitro* Daisy^{II} method, which is based on rumen fluid in the laboratory. When comparing the two methods, it became apparent that there were differences in the calibration of the degradation fractions. However, heat treatment in cake subjected to *in situ* could possibly increase the proportion of rumen non-degradable protein to small intestine if the heat strength would not impair amino acids.

Morama bean cakes appeared to offer higher DM and CP availability to rumen microbes compared to sunflower seed cake. However, *in vivo* validation is necessary, as secondary compounds in the bean cake may counterbalance some nutritional benefits. Additionally, the *in situ* method provided better reproducibility than the Daisy^{II} *in vitro* technique, despite some differences in calibration of degradation fractions.

Keywords: *in vitro* Daisy^{II} incubator; *in situ* rumen technique, *Morama* bean cake decorticated; *Morama* roasted bean cake; whole *Morama* bean cake; whole sunflower seedcake.

3.1 Introduction

The determination of dry matter (DM) and crude protein (CP) is an essential part of feed evaluation for ruminants; and the fact that protein is usually a limited but important nutrient. Therefore, sufficient quantification of DM and CP degradation in the rumen is of great interest to assess the nitrogen utilization efficiency of ruminants and accurately meet the animals' needs (Okon *et al.*, 2023). DM and CP degradation invariably occurs conveniently *in vivo* as it represents a real use in the animal. However, due to the differences between individual animals, this method is considered complex, time-consuming and error-prone (Stern *et al.*, 1997). This has necessitated alternative methods to be used in estimating DM and/or CP degradability values in ruminants.

For this reason, the method that could be used without simulation is the *in situ*, which uses a fistulated animal. In this protocol, bags are weighed and introduced into the rumen of cannulated animals at specific time points (0, 2, 4, 8, 12, 24, 48, 72, and 144 hours of incubation time) to measure and report feed losses in the bags and provides information about the rate and extent of feed degradation (Kitessa *et al.*, 1999). Due to the repeatability of the results, the method is usually used as a reference for degradation parameters in the rumen when fitted to appropriate nonlinear models (Ørskov and McDonald, 1979; GFE, 2022). These parameters are used in feed evaluation models to estimate nutritional value, nutrient supply and animal performance (Hackmann *et al.*, 2010). Although this technique could be used as a standard for comparing *in vitro* technique (Kitessa *et al.*, 1999), it has limitations that affect the objective estimation of protein degradation (Chrenkova *et al.*, 2019). *In situ* is laborious and requires a large number of analyses and rumen-cannulated animals; it also has problems associated washing losses and colonization of the residue by rumen microbes (Chrenkova *et al.*, 2019). These shortcomings associated with this technique

often lead to the use of *in vitro* methods, which are cheaper and easier to standardize. For example, the *in vitro* Daisy¹¹ technique (Ankom Technology, Macedon, NY, USA) allows estimates of DM and CP degradation with capability to screen large numbers of substrates (Adesogan, 2005; Dijkstra *et al.*, 2005). However, the criticism of this method (*in vitro* Daisy^{II}) compared to *in situ* relates to a possible alteration in the fermentation properties of the rumen fluid through collection, manipulation, dilution with buffer and the accumulation of end products during fermentations (Mould, 2003).

Therefore, the aim of the present study was to characterize the nutrient composition and evaluate the dry matter (DM) and crude protein (CP) degradability values of three different processed *Morama* bean cakes (MBCD, RMBC, and WMBC) and sunflower seed cake using *in vitro* and *in situ* techniques to compare the results of both methods; and to identify potential *Morama* bean cake that could be efficiently used as a nutritional supplement in the diet of ruminants.

The hypothesis was that the nutritional composition and kinetic degradability values of DM and CP of different processed *Morama* bean and sunflower seed cakes might be different due to the susceptibility of the feed ingredients to the techniques as determined by the use of *in vitro* and *in situ* rumen techniques.

3.2 Materials and Methods

3.2.1 Feedstuffs procurement and preparations

Tylosema esculentum (*Morama*) beans were bought from three locations in the Ntsokwe ploughing regions of Kweneng district, at the following sites: A (S 24° 01' 08.0" E24° 35' 16.0"), B (S 24° 00' 45.7" E24° 35' 31.9"), and C (S 23° 58' 49.3" E24° 38' 28.1"); at altitude of 1115 m above sea level. Sunflower seeds were purchased from the Botswana Agricultural Marketing Board (BAMB).

After purchasing, *Morama* beans were thoroughly mixed and placed in a 200-litre barrel. Sunflower seeds (WSSC) were also placed in a separate container. Four representative samples, each weighing 3 kg, were taken to the University of Botswana (UB) where they were processed into whole *Morama* bean cake (WMBC), decorticated *Morama* bean cake (MBCD), roasted *Morama* bean cake (RMBC), and whole sunflower seed cake (WSSC).

For decorticated *Morama* bean cake (MBCD), the shells were first removed by cracking them with a light wooden mallet. The peeled shells were then manually removed, leaving only the beans/nuts. The beans were then placed in a stainless-steel vessel.

For the roasted *Morama* bean cake, the beans were heated in a domestic oven (Defy 600 Series Gas, Caterlynx (PTY) LTD, Cape Town, RSA) at 250°C for 20 minutes. After cooling, the beans were separated from their shells by light blows with a mallet, while others were separated by hand because they had burst from the heat. Once ready, the beans/nuts were placed in a separate container.

The third and fourth samples of *Morama* beans (whole *Morama* bean cake) and sunflower seeds (whole sunflower seed cake) remained intact but were each placed in separate vessels.

Each feed ingredient sample was treated in an electric oil extractor (KK 20F Universal Oil Seed Press, Kern Kraft, Aqua Helios LTD, London, UK) for 30–60 minutes, resulting in more than 40% of the oil being pressed from the seeds/nuts. The oil cakes were placed in polyethylene bags and stored at 4°C in a rodent-free area at the Botswana University of Agriculture and Natural Resources (BUAN) until used for *in vitro* and *in situ* rumen studies.

3.2.2 Analysis of fatty acids from the oil seed press cakes

0.5 kg package of each oilseed (sunflower and *Morama*) was taken to the National Food Research Technology Center (NFRTC) for fatty acid profiling. Samples were extracted in triplicate using a modified method of AOAC (1996). Briefly, samples were directly converted to fatty acid methyl ester (FAME) and analyzed by capillary gas chromatography. HP 5890 Series II gas chromatograph (Hewlett Packard Co., Palo Alto, CA) equipped with a quartz glass capillary column (DB23 60 m 0.25 mm (0.25 m d.f.)). For quantification of the fatty acid content of the samples (in percent by weight), the synthetic fatty acids (typically C13:0, C19:0, C21:0 or C23:0) were added to the samples before extraction as internal standards and were quantified using ChemStation software (Agilent Technologies, Wilmington, USA). The carrier gas used was helium. The flame ionization detector temperatures were set at 260 °C, and the split ratio was 80:1. The temperature was initially set at 140°C for three minutes and later increased to 240°C for one minute, held for 10 minutes and finally decreased to 140°C and held for a fraction of a minute.

3.2.3 Chemical and mineral analysis

The feeds tested were whole *Morama* bean cake (WMBC), *Morama* bean cake decorticated (MBCD), roasted *Morama* bean cake (RMBC), and whole sunflower seed cake (WSSC). Samples were prepared in triplicate and processed in a Wiley mill (Thomas Wiley Mill Model 4; Thomas Scientific USA, Swedesboro, NJ) with a 2-mm sieve for the *in situ* assay and with a 1-mm sieve for *in vitro* assay, lightly ground and placed in a collapsible desiccator (Absorb King Co., Ltd, Shenzhen City, Guangdong, China) and dried at 60 °C for 48 h.

The feed samples were analyzed in triplicate for dry matter (DM), crude protein (CP), and ash (AOAC, 2003). The ether extract (EE) was analyzed with diethyl ether using the ANKOM XT10 fat analysis system (AOAC, 2003). Neutral detergent fiber (NDF) was determined (Martens, 2002) after treatment with α -amylase and sodium sulphite using the ANKOM220 Fibre Analyzer (ANKOM Technology®, Macedon, NY, USA). Acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were sequentially determined after NDF according to Robertson and Van Soest (1981). The measurements of NDF, ADF, and lignin were corrected by subtracting values obtained from blanks. According to AOAC (1997), the Kjeldahl technique was used to determine nitrogen (N) content, which was then multiplied by a factor of 6.25 to estimate crude protein in the feeds. Non-fiber carbohydrates (NFC) were determined by difference, where: $NFC = 100 - (\%neutral\ detergent\ fiber + \%crude\ protein + \%fat + \%ash)$. The metabolizable energy (ME) content of feed ingredients (MJ/kg DM) was determined from the chemical composition using the lignin-based approach proposed by NRC (2001). Cellulose content was determined as the difference between acid detergent fiber (ADF) and acid detergent lignin (ADL), while hemicellulose was determined as the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF).

According to Porter *et al.* (1986), the butanol-HCl-Fe technique was used to measure condensed tannin (CT), with results presented as leucocyanidin equivalents. Briefly, the butanol-HCl reagent (butanol-HCl 95:5 v/v) was prepared by mixing 950 mL of N-butanol with 50 mL of concentrated HCl (37%). The iron reagent (2% ferric ammonium sulfate in 2N HCl) was prepared by dissolving 2.0 g of ferric ammonium sulfate in 2N HCl (16.6 mL of concentrated HCl made up to 100 mL with distilled water to make 2N HCl).

The feed samples were also analyzed for the micro minerals: iron, selenium, manganese, copper, and zinc, as well as macro minerals (calcium, phosphorus, potassium, magnesium, and sodium) using an inductively coupled plasma-optical emission spectrometer (Perkin-Elmer, OPTIMA - 3300 RL).

3.3 Experiment 1

3.3.1. *In vitro* degradability assay with Daisy^{II} incubator

For *in vitro* determination, a Daisy^{II} incubator (ANKOM Technology, Macedon, NY, USA), consisting of a thermostatic chamber (39 ± 0.5 °C) with 4 rotating jars, was used to test the digestibility or degradability of DM and CP according to Tilley and Terry (1963) modified by Goering and Van Soest (1970) as described by Ammar *et al.* (1999). The rumen fluid used was collected as shown below:

3.3.2 Donor cows' management and rumen collection

Rumen fluid from three Holstein-Friesian cows (average weight: $550 \text{ kg} \pm 1.05 \text{ kg}$) was collected and used for an *in vitro* degradability test at the Agricultural Research Council (ARC), Irene, South

Africa. Every day at 8:30 a.m. and 4:30 p.m., animals were fed 12 kg DM diet consisting of *Eragrostis rigidior* hay in combination with alfalfa haylage (containing 120 and 410 g/kg DM of CP and NDF, respectively). The Agricultural Research Council (ARC) good practice criteria for the use of animals in research were followed, including allowing animals' free access to water and mineral salts and providing care in accordance with these standards. The necessary approval (Ref. No. APIEC16/023) for the collection of rumen fluid from cows was requested and granted by the Agricultural Research Council (ARC) of the Animal Ethics Committee of the Republic of South Africa. To limit the variance in rumen microbial activity, rumen fluid was collected 2 h before morning feeding and transported to the laboratory under continuous flushing with CO₂.

3.3.3 Preparation of inoculum and rumen fluid for *in vitro* degradability assay

The inoculum was prepared by mixing the rumen fluid and particles in a household blender for three minutes while continuously purging with carbon dioxide, and then filtering through four layers of cheesecloth while continuing to purge with CO₂. According to Navarro-Villa *et al.* (2011), the rumen fluid was combined with a buffer in a ratio of 1:4 (v/v). The buffer solution used was a combination of solution A (1330 mL/incubation vessel; 10 g KH₂PO₄, 0.5 g MgSO₄·7H₂O, 0.5 g NaCl, 0.1 g CaCl₂·H₂O and 0.5 g CH₄N₂O (carbamide or urea) in 1 L deionized water) and solution B (266 mL/incubation jar; 15 g Na₂CO₃ and 1 g Na₂S·9H₂O in 100 ml deionized water). The feed samples (WMBC, MBCD, RMBC, and WSSC) were ground to pass through a 2 mm sieve, weighed into nylon bags with a pore size of 42 μ m and internal dimensions of 6 × 5 cm, and thermally sealed. A total of 2 L each of buffer solution and rumen inoculum were added to the digestion vessel and left to stand in a water bath at 39 °C for up to 24 hours. Before each digestion run, a buffer was created by heating solutions A and B each to 39 °C and then adding 20 mL of

each to 1 L of solution A. Small amounts of solution B were added to the buffer solution to lower the pH to 6.8.

Two consecutive incubations were performed with 2 incubations x 2 rotating jars x 4 feed ingredients x 2 replicates, yielding a total of 8 values per feed ingredient plus 8 empty bags used as blank samples. The feed samples weighing 1.250 ± 0.001 g per bag were weighed into the reduced nylon bags. The bags were then placed into the vessels filled with buffered rumen fluid as described above.

3.3.4 Experimental Design

The *in vitro* experiment was conducted according to a complete factorial design (4 inocula x 4 substrates), repeated two times (2 periods) (n = 32). At 2, 4, 6, 12, and 24 hour intervals; 4 bags of each feed were incubated in a Daisy^{II} apparatus under constant anaerobic conditions. The jars contained four samples in each bag holder set in a random order. After 24 hours, the nylon bags were individually removed from the chamber and then washed under running water for 30 minutes. The bags were dried in a forced air oven at 60°C for 48 hours, cooled in a collapsible desiccator, and then allowed to dry for 45 minutes.

3.3.5 Computation of disappearance parameters

The resulting weight and analytical differences between pre-and post-incubations at the times, 2, 4, 6, 12, and 24 h, and their assessment as a percentage of the initial value, were used to calculate the degradability values for the dry matter (DM) and crude protein (CP) as shown below:

$$(i) \quad \text{Dry matter degradability}(\%) = \frac{DM - DM_{rfd}}{DM} \times 100$$

$$(ii) \quad \text{Crude protein degradability}(\%) = \frac{CP - CP_{rfd}}{CP} \times 100$$

Where, DM_{rfd} is the dry matter after incubation, DM is the initial dry matter before incubation and CP_{rfd} is the crude protein content after rumen fluid incubation; CP is the crude protein content of the sample before adding rumen fluid incubation.

The crude protein content of the sample was determined using the Kjeldhal method. The difference between the initial CP content and the CP content after incubation with the rumen fluid was considered as a measure of the disappearance of CP or the degradable amount.

Using the computer program NEWAY reported by Ørskov and McDonald (1979), the *in vitro* degradability constants of DM and CP were calculated using nonlinear least squares models. Nutrient components were estimated at rumen solid effluent rates of 0.02, 0.05, and 0.08/h. According to Ørskov and McDonald (1979), the formulas are $EDDM = (a + b) * k_d / (k_d + kp)$, and $EDCP = (a + b) * k_d / (k_d + kp)$ were used to calculate the effective DM and CP degradabilities. In this case, ED stands for effective degradability, a represents the soluble fraction, b represents the potentially degradable but insoluble fraction, u represents the non-degradable residual pools ($u = 100 - a - b$), k_d represents the fractional degradation rate of fraction b and kp represents the transit or outflow rate, which is assumed to be either 0.02, 0.05 or 0.08/h.

3.3.6 Statistical analysis

The NLIN method of the Statistical Analysis System for Windows software, 9.4 (SAS, 2012) was used to fit the disappearance kinetics of DM and CP. One-way analysis of variance (ANOVA) was performed and noniterative least squares method was used.

Correlation analyses of the data were also used to determine the possible effects of the physical and chemical properties of the oilseed cakes on their rumen degradation. Multivariate regression equations for predicted values (a , b , u , k_d , and ED), DM, and CP degradation characteristics were derived using a stepwise technique (backward elimination). The independent and dependent variables of the stepwise approach were determined from the actual results of degradation rates, other constants, and chemical composition.

Regressions related to parameter solubility and/or insolubility were compared using a test based on indicator variables (Neter *et al.*, 1990). The mean differences for the noniterative least squares process was considered significant at $P < 0.05$. $P < 0.15$ was used as the entry and exclusion threshold for parameters that had no interaction with the chemical components. The equation with the highest R^2 and lowest P -value was selected as the most suitable model.

3.4 Experiment 2

3.4.1 *In situ* dry matter and crude protein degradability

The *in-situ* incubation study took place at the Department of Agricultural Research (DAR) experimental dairy farm in Gaborone, Botswana. This was conducted after approval from the Animal Care and Use Committee of BUAN Animal Scientists (BUAN-AEC-2021-04). Three rumen-cannulated Tswana steers with an initial body weight (BW) of 573 ± 29.4 kg (mean $\pm$ standard deviation) were used for the experiment. The animals were housed in a 20 x 20 m kraal for 5 days to acclimate to handling, as opposed to the regular 14-day schedule (Van Soest, 1994), since they were already accustomed to the surroundings and the feed.

During the adaptation phase, steers were offered two equal portions of the total mixed ration (TMR) twice daily, at 8:30 a.m. and 4:30 p.m. Steers were fed maintenance level TMR (0.5% BW) consisting of 64.2% cracked white maize kernels, 20.9% ground alfalfa, 10.3% wheat bran, 4.1% molasses powder, 0.31% dicalcium phosphate and 0.21% coarse salt. Diets were formulated to provide 12% CP and approximately 3.47 MJ/kg DM (NFC = 78.8%) energy. The animals had free access to water between feeding times. The chemical composition of the total mixed ration (TMR) was analyzed for DM and CP using the method of AOAC (1990).

3.4.2 Preparation of the feed ingredients

In situ polyester nylon bags (190 x 45 mm, 40 μ m pore size; 100% polyester, R102 Marvelaire White, N. Erlanger, Blumgardt, 1450 Broadway, NY, USA) were used in this study (Ørskov & McDonald, 1979). The ratio of sample size to bag surface area was 17.5 mg/cm², which was within the recommended range (Nocek, 1988). Approximately 4 x 5 g bags of the dried test feed (placed

in the nylon bags) were placed in the liquid phase of the rumen of each of the three Tswana steers. The bags were incubated in three replicates at 0, 2, 4, 6, 8, 16, 24, and 48 hours in the rumen of each of the three steers, with the exception of the zero-hour bags, which were soaked in lukewarm water (Nocek, 1988) for 30 minutes before being hand-washed to estimate the proportion that disappeared quickly (*a*). Each time the bags were removed, each steer's rumen pH was measured using a glass electrode pH meter (HANNA 8520 Instruments QA, Supplies, LLC, 1185 Pineridge Road, Norfolk, Virginia, USA) and approximately one liter (1 L) of rumen fluid was collected. After washing, the nylon bags were dried in a forced air oven at 60°C for 72 hours, while the rumen fluid was filtered through four layers of cheesecloth to remove debris and protozoa (Menke and Steingass, 1988). The final rumen filtrate was prepared according to the method of Promkot *et al.* (2007), collected, centrifuged, and bottled. Five (5) mL of 1 M H₂SO₄ were added and stored at -20°C for later analysis of ammonium nitrogen (NH₃-N) according to AOAC (1990). The residues from a set of dried bags at each time point of a given treatment were pooled and analyzed for dry matter and crude protein (AOAC, 1990). Therefore, the calculation was carried out according to Osuji *et al.* (1993) as shown:

$$\text{DMD/CP} = ((\text{BW} + \text{S1}) - (\text{BW} + \text{RW})) (\text{S1} \times \text{DM}) \times 100$$

Where, BW – bag weight; S1 – sample weight; RW – residue weight; DM – dry matter concentration of the original sample.

Thus, the resulting data from the disappearance of DM and CP due to rumen microbial utilization of each of the four feed ingredients (*in situ*) were then fitted (according to Equation 1) to a non-linear regression using the Gauss-Newton method (Neter *et al.*, 1985) with the SAS Users Guide: Statistics (SAS Inst., 2012; Cary, NC, USA).

$$\text{DM or CP} = a + b (1 - e^{-kt}) \quad \text{(Equation 1)}$$

Where, a is the rapid soluble fraction (% measure of the parameters, DM, and CP); b is the slowly degradable fraction (% measure of the parameters, DM, and CP); k_d is the fractional disappearance rate (%/h) of b ; t is the time of ruminal incubation (0, 2, 4, 6, 8, 16, 24 and 48 h), e – natural logarithm (2.718).

The parameters a , b , u and k_d were determined using nonlinear methods from SAS (Statistical Analyzes Systems, Version 9.4, (2012)). The estimated loss of rumen dry matter or crude protein (effective degradability) was calculated using the formula of Batajoo and Shaver (1994).

$$ED = a + (b * k_d)/(k_d + kp) \quad \text{(Equation 2)}$$

Where, a , b , k_d , kp , are as defined previously in **Experiment 1**.

The kinetic degradation parameters for DM and CP were at fractional discharge rates (kp) of 0.02, 0.05 and 0.08/h (AFC, 1993). Turnover rates (kp) were based on maintenance feeding of adult or non-productive animals, medium-productive animals or fast-growing animals (lambs, calves, goats) or dairy cows (AFRC, 1993).

3.4.3 Experimental Design and Statistical Analysis

Four feed ingredients were replicated three times and randomly assigned to the three steers in a completely randomized design matrix (CRD) (4 feed ingredients x replicated 3 times, 3 steers) over 2 periods. The data were subjected to analysis of variance according to the model:

$$Y_{ijk} = \mu + D_i + A_j + P_k + e_{ijk}$$

Where: Y_{ijk} represents the dependent variable; μ is the overall mean; D_i is the feed ingredient effect ($i = 1$ to 4); A_j the animal effect ($j = 1$ to 3); P_k , the period effect ($k = 1$ to 2) and e_{ijk} is the random error.

Rumen disappearance data were analyzed using SAS (2012), considering a steer as the random effect and feed as the fixed effect. Differences between feed degradations were determined using the *LSMeans* statement with the PDIFF option and were considered significant in the *F*-test ($P < 0.05$). The kinetic parameters of DM and CP disappearance over time were predicted according to Ørskov and McDonald (1979) by fitting data from the feed residues in the nylon bags with a non-linear equation.

The relationships between the parameter fractions and the chemical composition of the oilseed press cake were evaluated using correlation coefficients. Multivariate regression equations for predicted values (a , b , u , k_d , and ED), DM and CP degradation properties were derived using a stepwise technique (backward elimination). Variables (independent and dependent) used in the addition or subtraction of the stepwise approach were determined from chemical composition and *in situ* DM and CP degradability tables. Entry and exclusion criteria were set at $P < 0.15$. The equation with the highest (R^2) and lowest *P*-value was chosen as the best-fit model.

3.4.4 Comparison of *in vitro* Daisy^{II} and *in situ* techniques

The differences between *in vitro* and *in situ* estimates of degradation kinetics (a , b , u , and k_d), including effective DM and CP degradation at assumed rumen outflow rates of 0.02, 0.05, and 0.08/h, were tested using a pooled *t*-test or the Satterthwaite approximation of the *t*-test applied according to the folded *F*-test. The degradation kinetics were expressed as a degradation quotient

(deQ) according to Okon *et al.* (2023): $\text{deg } Q = (\text{degradation kinetics including effective degradability}_{in vitro} - \text{degradation kinetics including effective degradability}_{in situ}) / \text{degradation kinetics including effective degradability}_{in situ}$ or $\text{deg } Q = \frac{(ED_{in vitro} - ED_{in situ})}{ED_{in situ}}$

Technique Model comparison: The goodness-of-fit was evaluated using the coefficient of determination (R^2) (Lopez *et al.*, 2004) and *F* ratio test (Motulsky and Ransnas (1987).

3.5 Results

3.5.1 Nutritive value of feed ingredients

The nutritional composition of the protein sources used for *in vitro* and *in situ* rumen degradability are listed in Tables 3.1 and 3.2, respectively. There were differences between the four dietary components in ADL value ($P < 0.05$) and cellulose content ($P < 0.01$). WMBC and WSSC did not differ ($P > 0.05$) for CP, ADF, and NDF content, but both differed from RMBC and MBCD for the same components ($P < 0.05$). WMBC and MBCD did not differ ($P > 0.05$) for DM, EE, and hemicellulose content, but both differed from RMBC and WSSC for the same components ($P < 0.05$). WMBC and RMBC did not differ ($P > 0.05$) for CT, ash, and OM contents, but both differed from MBCD and WSSC for the same components ($P < 0.05$). RMBC and WSSC did not differ ($P > 0.05$) for NFC and ME content, but both differed from WMBC and MBCD for the same components ($P < 0.05$).

Table 3.1: Chemical composition (%) of decorticated and undecorticated seedcakes of *Morama* and sunflower cakes

Constituents	WMBC	MBCD	RMBC	WSSC	SEM
Dry matter	92.4 ^c	92.4 ^c	94.8 ^b	95.9 ^a	1.02
Organic matter	82.6 ^c	86.4 ^b	84.4 ^c	90.9 ^a	0.43
Crude protein	21.9 ^c	32.9 ^b	39.2 ^a	22.2 ^c	4.90
Ether extract	8.5 ^a	8.4 ^a	7.1 ^b	8.5 ^a	0.04
Acid detergent fibre	10.6 ^c	21.8 ^a	18.1 ^b	10.5 ^c	3.25
Neutral detergent fibre	26.3 ^c	26.2 ^c	31.4 ^b	53.1 ^a	7.39
Acid detergent lignin	1.3 ^d	1.8 ^c	2.1 ^b	2.7 ^a	0.34
Ash	9.8 ^a	6.0 ^b	10.4 ^a	5.0 ^b	1.56
Non fibre carbohydrate	41.2 ^a	33.6 ^b	18.3 ^c	18.9 ^c	0.55
Condensed tannin	4.1 ^a	1.6 ^c	4.0 ^a	2.4 ^b	0.07
Cellulose	9.6 ^c	15.4 ^b	16.4 ^a	8.3 ^d	2.35
Hemicellulose	25.0 ^c	24.1 ^c	29.8 ^b	53.4 ^a	7.86
ME(MJ/kg DM)	6.92 ^b	6.89 ^b	10.87 ^a	9.65 ^a	1.00

^{a, b, c} means along the same rows bearing different superscripts are significantly different ($P < 0.05$); WMBC – whole morama bean cake; MBCD – morama bean cake decorticated; RMBC – roasted morama bean cake; WSSC – whole sunflower seed cake; DM– dry matter, SEM – standard error of mean

The calcium content of all cakes (0.2% DM) was comparable ($P > 0.05$), except for the *Morama* bean cake decorticated, which had a lower content (0.1% DM; $P < 0.05$). The P, K, and Mg contents of the *Morama* cakes were 0.4, 0.9, and 0.1% for WMBC, MBCD, and RMBC, respectively. The whole sunflower seed cake contained significantly more macrominerals ($P < 0.05$) than the legume cakes. There was no difference in Na content between cakes ($P > 0.05$).

Table 3.2: Mineral analysis (%) of decorticated and undecorticated seedcakes of morama and sunflower

Constituent	WMBC	MBCD	RMBC	WSSC	SEM
Calcium (Ca)	0.2 ^a	0.1 ^b	0.2 ^a	0.2 ^a	0.02
Phosphorus (P)	0.4 ^c	0.5 ^b	0.1 ^d	0.7 ^a	0.1
Potassium (K)	1.0 ^a	0.9 ^{ab}	1.1 ^a	1.0 ^a	0.03
Magnesium (Mg)	0.3 ^b	0.4 ^a	0.1 ^c	0.3 ^b	0.1
Sodium (Na)	0.1	0.1	0.1	0.1	0.01
Iron (Fe)	0.1	0.001	0.001	0.001	0.01
Manganese (Mn)	0.9 ^c	1.2 ^b	0.3 ^d	2.2 ^a	4.1
Selenium (Se)	7.3 ^a	7.2 ^c	6.8 ^d	7.2 ^b	0.1
Zinc (Zn)	0.002	0.003	0.001	0.007	0.001

Copper (Cu)	0.002	0.002	0.002	0.003	0.003
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WMBC- whole *Morama* bean cake; MBCD-*Morama* bean cake decorticated; RMBC-roasted *Morama* bean cake; WSSC-whole sunflower seedcake; SEM-standard error mean; ^{a, b, c, d} means along the same rows bearing different superscripts are significantly different ($P < 0.05$).

3.5.2 Oilseeds (sunflower and *Morama*) fatty acid profile

The fatty acid (FA) profile of oilseeds is shown in Table 3.3. The results show that sunflower and *Morama* seeds are characterized by low saturated fatty acid (SFA) content. *Morama* beans have the highest average palmitic and stearic acid content (1.22 and 2.51%, respectively). Additionally, *Morama* beans have the highest average oleic acid content than sunflower seeds. Both (*Morama* and sunflower) oilseeds have a comparable linoleic acid content ($P > 0.05$). *Morama* beans also contained trace amounts of arachin and eicosanoid oils, while sunflowers contained none of these oils.

Table 3.3: Fatty acids derived from sunflower and *Morama* beans (% of total fatty acid)

Fatty acid	Sunflower seedcake (%) (n =3)	<i>Morama</i> bean cake (%) (n =3)
Palmitic acid (C16:0)	0.65 ± 0.10	2.51 ± 0.20
Stearic acid (C18:0)	0.32 ± 0.05	1.22 ± 0.15
Oleic acid (C18:1)	1.24 ± 0.12	9.42 ± 0.25
Linoleic acid (C18:2)	4.94 ± 0.30	4.99 ± 0.28
Arachidic acid (C20:0)	-	0.46 ± 0.05
Eicosenoic acid (C20:1)	-	0.71 ± 0.08

Note: “-” indicates data not available or not detected.

3.5.3 *In Vitro* Rumen Degradability

3.5.3.1. *In vitro* disappearance for DM and CP of cakes

Figures 3.1 and 3.2 show the disappearance values of DM and CP for sunflower and *T. esculentum* cakes (MBCD, WMBC and RMBC). According to Figure 3.1, the mean DM degradability values for WMBC, MBCD, RMBC, and WSSC were 36.5%, 69.0%, 51.3%, and 32.9%, respectively, and were significantly different ($P < 0.05$). Within 24 hours of incubation, the mean CP degradability of the oilseed press cakes (WMBC, MBCD, RMBC, and WSSC) were 49%, 97.2%, 44.6%, and 90%, respectively.

The crude protein degradability values for WMBC and RMBC did not exceed 50% at 12 hours, while MBCD and WSSC were 96.3% and 89.7%, respectively (Figure 3.2). After 24 hours of incubation, the CP degradability values of MBCD and WSSC were 97.2% and 90%, respectively. The disappearance of DM and CP increased with incubation time for all feed components (0-24 hours), but the increase was slow for WMBC and RMBC.

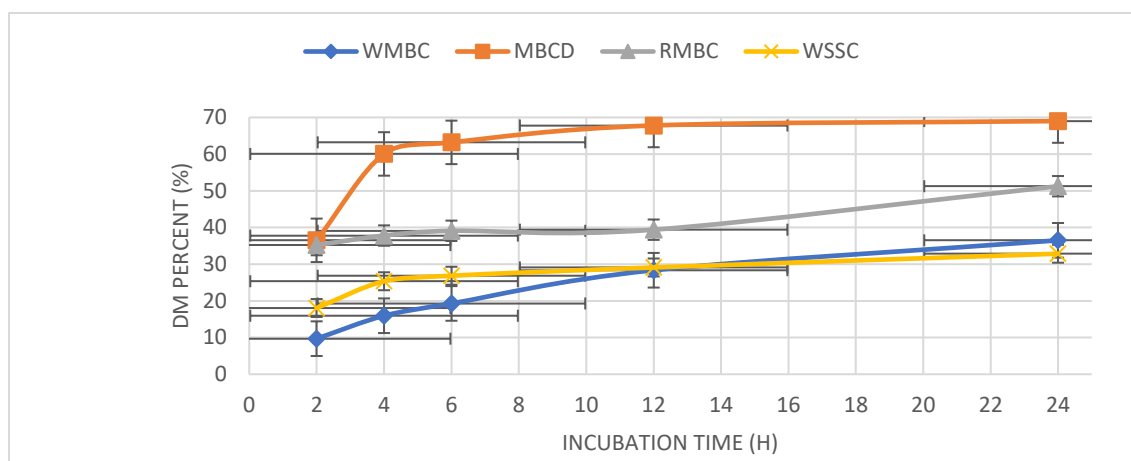


Figure 3.1: DM degradability of feed ingredients (WMBC, MBCD, RMBC, and WSSC) over incubation time in vitro technique

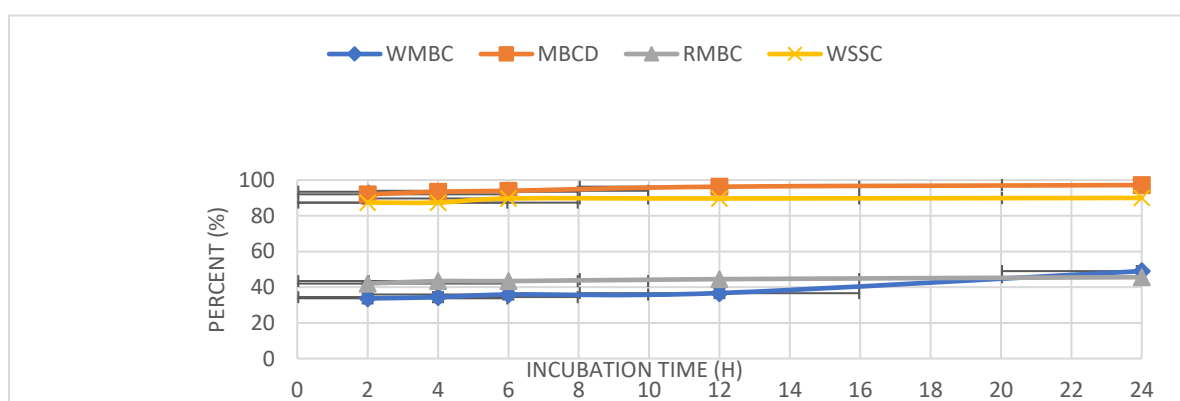


Figure 3.2: CP degradability of feed ingredients (WMBC, MBCD, RMBC, and WSSC) over incubation time in vitro technique

3.5.3.2 *In vitro* DM and CP Degradation characteristics

Table 3.4 shows the *in vitro* DM and CP degradation profiles of *T. esculentum* (WMBC, MBCD and RMBC) and sunflower seed cake. There was no evidence of a lag time in the degradation values of the model used. The mean values of the effective degradability of the dry matter (DM) showed a small scatter: mean = 24.3% (CV = 2.0%) for whole *Morama* bean cake (WMBC), mean = 44.7% (CV = 2.0%) for decorticated *Morama* bean cake (MBCD), mean = 24.2% (CV = 3.5%) for roasted *Morama* bean cake (RMBC), and mean = 36.0% (CV = 1.7%) for whole sunflower

seed cake (WSSC). These values did not differ significantly ($P < 0.05$) in terms of the coefficient of variation (CV).

The dry matter degradation kinetic estimates (a , b , u , and k_d) showed moderate variability (Table 3.4). The non-degradable (u) content had the highest mean values for whole *Morama* bean cakes (60.2%), while it was lowest for decorticated *Morama* bean cake (35.5%). The mean effective DM degradability (EDDM) of WMBC and RMBC was lowest ($P < 0.05$) at estimated rumen outflow rates (0.02, 0.05, and 0.08/h), while MBCD had the highest rates per hour ($P < 0.05$).

For *in vitro* CP, the kinetic degradation fractions showed moderate variability, except for the nondegradable fraction in MBCD (CV = 74.1%), which varied between 0.1% and 3% of total N. However, the relative spread for effective CP degradation in the rumen was lowest in WMBC. The mean effective crude protein degradability (EDCP) of MBCD was the highest (76.6%; $P < 0.05$) among all feeds, while the lowest was for WMBC (21.0%; $P < 0.05$) at the same assumed ruminal discharge rates

For *in vitro* crude protein (CP), the kinetic degradation fractions showed moderate variability, except for the nondegradable fraction in decorticated *Morama* bean cake (MBCD) (CV = 74.1%), which varied between 0.1% and 3% of total nitrogen (N). However, the relative spread for effective CP degradation in the rumen was lowest in whole *Morama* bean cake (WMBC). The mean effective crude protein degradability (EDCP) of MBCD was the highest (76.6%; $P < 0.05$) among all feeds, while the lowest was for WMBC (21.0%; $P < 0.05$) at the same assumed ruminal discharge rates.

Table 3.4: Least squares means of *in vitro* degradation characteristics of dry matter (DM) and crude protein (CP) in *T. esculentum* (MBCD, RMBC and WMBC) and sunflower seed cakes (WSSC)

Item	WMBC			MBCD			RMBC			WSSC		
Dry matter	Mean	C.V%	Range	Mean	C.V (%)	Range	Mean	C.V(%)	Range	Mean	C.V(%)	Range
<i>a</i> (%)	3.50	7.8	3.1-3.9	24.2	4.3	22.5-25.1	11.7	8.00	10.2-13	21.9	4.2	20.1-22.9
<i>b</i> (%)	36.3	3.3	34.9-38.3	40.3	3.9	37.7-42.9	32.9	2.90	31.9-34.1	32.9	1.7	32.1-33.5
<i>u</i> (%)	60.2	2.1	57.9-61.7	35.5	5.4	32-37.4	55.3	3.20	53-57.7	45.2	2.7	43.6-47.6
<i>kd</i> (%/h)	0.18	5.6	0.17-0.2	0.19	8.7	0.18-0.21	0.13	2.70	0.13-0.14	0.18	3.3	0.17-0.19
<i>EDM</i> ₂ (%)	31.5	3.9	30.2-33.9	51.7	3.5	49.3-54.9	31.9	7.80	26.7-34.5	43.2	2.6	41.1-44.7
<i>EDM</i> ₅ (%)	24.0	1.2	22.9-25.3	44.2	1.2	42.2-45.1	23.5	1.70	20.3-24.1	35.8	1.60	34.2 - 37.3
<i>EDM</i> ₈ (%)	17.5	1.03	16.5-19.2	38.2	1.4	36.7- 40.1	17.3	0.96	16.6 -18.9	29.1	1.02	27.8-30.3
Crude Protein												
<i>a</i> (%)	8.60	4.6	7.9-9	5.6	12.3	4.7-6.6	11	5.6	10.1-12.2	5.3	4.9	4.9-5.6
<i>b</i> (%)	32.8	1.7	31.9-33.4	93.1	0.9	91.9-94.3	35.3	3.1	33.4-36.7	87.9	1.8	85.3-90.1
<i>u</i> (%)	58.6	1.4	57.7-60	1.3	74.1	0.1-3	53.7	2.8	51.1-55.7	6.8	26.5	4.5-9.8
<i>k_d</i> (%/h)	10.0	2.3	0.09-0.095	30	8.2	0.25-0.32	31	5.8	0.29-0.34	21	18.9	0.17-0.28
<i>EDCP</i> ₂ (%)	27.6	2.3	26.7-28.5	84.6	1.9	81.4-86.5	40.1	3.5	38-42.3	75.8	4.7	71.1-81.5
<i>EDCP</i> ₅ (%)	20.1	0.1	19.1-21.5	77.1	1.7	76.6-80.4	38.3	1.5	35.5-39.1	68.6	1.8	66.8-71.3
<i>EDMCP</i> ₈ (%)	15.2	0.1	13.1-17.4	68.2	1.8	66.3-70.6	29.5	1.4	27.8-31.2	61.4	1.6	58.8-62.6

WMBC - whole *Morama* bean cake; MBCD- *Morama* bean cake decorticated; RMBC- roasted *Morama* bean cake; WSSC-whole sunflower seed cake; CV- coefficient of variation; *a*, *b*, and *u* represent soluble, slowly degradable, and undegradable fractions, respectively; *k_d*- fractional degradation rate of fraction *b*; *EDM*₂, *EDM*₅ and *EDM*₈ – effective dry matter degradability at 0.02, 0.05 and 0.08/h ruminal passage rate ; *EDCP*₂, *EDCP*₅ and *EDCP*₈- effective crude protein degradability at 0.02, 0.05, and 0.08/h ruminal passage rate.

3.5.3.3 Correlation between *in vitro* dry matter degradation and chemical composition

Table 3.5 provides correlation coefficients for dry matter of *T. esculentum* (MBCD, WMBC, and RMBC) and sunflower seed cakes between chemical composition and *in vitro* degradation properties. The DM degradation properties of the feeds were related to their *a* in MBCD, WMBC, RMBC and WSSC ($r = -0.327$, $r = 0.469$, $r = -0.309$ and $r = -0.537$, respectively; $P < 0.05$), *b* in MBCD, WMBC, RMBC and WSSC ($r = 0.496$, $r = 0.644$, $r = 0.729$, $r = -0.417$, respectively; $P < 0.05$). ADF, ADL, cellulose and hemicellulose were all related to the non-degradable (*U*) fraction in MBCD ($r = 0.816$, $r = 0.792$, $r = -0.885$ and $r = -0.675$, respectively; $P < 0.05$) and the DM with non-degradable (*U*) for WMBC and RMBC ($r = -0.665$ and $r = -0.662$, respectively; $P < 0.05$). However, a high positive correlation of effective degradability (ED) with chemical components was found in the decorticated *Morama* bean cake (MBCD). The fractional degradation rate (k_a) was related to hemicellulose ($r = -0.650$; $P < 0.05$) in decorticated *Morama* bean cake (MBCD) and cellulose ($r = -0.567$; $P < 0.05$) in whole *Morama* bean cake (WMBC). Acid detergent lignin (ADL), hemicellulose, and condensed tannins (CT) ($r = 0.840$, $r = 0.700$, and $r = 0.530$, respectively; $P < 0.05$) in roasted *Morama* bean cake (RMBC) were more strongly related to the feed fractional degradation rate. In addition, the acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, and condensed tannins (CT) in whole sunflower seed cake (WSSC) ($r = 0.460$, $r = 0.858$, $r = 0.568$, and $r = -0.563$, respectively; $P < 0.05$) were found to be strongly associated with the feed parameters. There were positive correlations between average effective DM degradation (EDDM) with the cell wall contents (NDF, ADF, Cel. and Hemi) of MBCD and ADL of the RMBC.

Table 3.5: Correlation coefficients between chemical composition and in vitro degradation characteristics of dry matter of *T. esculentum* (MBCD, WMBC, RMBC) and sunflower seed cakes (WSSC)

	MBCD					WMBC				
	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	ED	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	ED
DM	-0.327	0.496*	-0.181	-0.032	0.085	0.469*	0.644*	-0.665*	0.125	0.547*
ADF	-0.285	-0.773*	0.816*	0.232	-0.562*	-0.395	0.113	-0.020	0.139	0.089
ADL	0.442	-0.642*	0.792*	0.130	-0.546*	-0.181	-0.033	0.070	0.183	-0.079
CEL	-0.312	-0.833*	0.885*	0.100	-0.666*	0.092	0.231	-0.196	-0.567*	0.016
HEM	0.886*	0.223	-0.675*	-0.650	0.862*	-0.231	0.491*	-0.408	-0.271	0.200
CT	0.062	0.014	-0.012	-0.021	0.040	0.116	0.038	-0.010	-0.022	0.182
	RMBC					WSSC				
	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	ED	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	ED
DM	0.309	0.729*	-0.662*	-0.048	0.601*	-0.537*	-0.417	0.441	-0.424	-0.479*
ADF	-0.314	-0.103	0.220	0.207	-0.235	-0.213	-0.128	0.215	0.460*	-0.182
ADL	0.600*	-0.189	0.418	0.84*	-0.546*	0.336	-0.162	0.176	0.858*	-0.223
CEL	-0.210	-0.594*	0.432	0.110	-0.148	-0.134	-0.082	0.062	0.568*	-0.057
HEM	0.720*	0.800*	-0.812*	-0.70*	0.229	0.192	0.513*	-0.152	-0.147	0.371
CT	0.402	0.283	-0.364	-0.53*	0.164	0.224	0.337	-0.316	-0.563*	0.276

DM-dry matter; ADF – acid detergent fibre; ADL- acid detergent fibre; Cel – cellulose; Hemi- hemicellulose; CT- condensed tannin; MBCD – *Morama* bean cake decorticated; WMBC – whole *Morama* bean cake; RMBC – roasted *Morama* bean cake; WSSC-whole sunflower seed cake; *significant at $p < 0.05$, *a*, *b*, and *u* represent soluble, slowly degradable, and undegradable fractions, respectively ED - effective crude protein degradability at 0.02, 0.05, and 0.08/h ruminal passage rate.

3.5.3.4 Correlation between *in vitro* crude protein degradation and chemical composition

The correlation coefficients between chemical composition and *in vitro* degradation characteristics of the crude protein of *T. esculentum* (MBCD, WMBC, and RMBC) and sunflower seed cakes are shown in Table 3.6. All feeds showed low or non-significant correlation between the rapid solubility (*a*) of crude protein and crude protein content, while the crude protein level of WSSC was moderately negatively correlated with fraction *a* ($r = -0.659$; $P < 0.05$). However, the crude protein content of MBCD and WMBC was moderately associated with the slowly degradable fractions, *b* ($r = 0.699$ and $r = 0.573$, respectively; $P < 0.05$).

The crude protein contents of MBCD, WMBC, and WSSC were moderately and negatively related to the non-degradable fraction u ($r = -0.575$, $r = -0.574$, and $r = -0.526$, respectively; $P < 0.05$). The fractional degradation rate (k_d) was associated with CP, ADF, and cellulose ($r = -0.768$, $r = 0.566$, and $r = 0.591$) of MBCD; ADF, ADL, cellulose, and hemicellulose ($r = 0.540$, $r = 0.538$, $r = 0.542$, and $r = 0.748$) from WMBC; ADF, ADL, and cellulose ($r = 0.50$, $r = 0.729$, and $r = 0.632$) of RMBC; and cellulose ($r = 0.663$) from WSSC.

The effective degradability (ED) of decorticated *Morama* bean cake (MBCD) was strongly and positively associated with crude protein (CP) ($r = 0.785$) and moderately but negatively with acid detergent fiber (ADF) and cellulose ($r = -0.569$ and $r = -0.497$). The ED of whole *Morama* bean cake (WMBC) was associated with CP, ADF, ADL, and hemicellulose ($r = 0.466$, $r = -0.493$, $r = -0.594$, and $r = 0.577$, respectively; $P < 0.05$). The ED of roasted *Morama* bean cake (RMBC) was related to ADL, hemicellulose, and condensed tannins ($r = -0.521$, $r = 0.613$, and $r = -0.477$, respectively; $P < 0.05$). The ED of whole sunflower seed cake (WSSC) was associated with CP, cellulose, and CT ($r = 0.501$, $r = 0.700$, and $r = -0.782$, respectively; $P < 0.05$).

Table 3.6: Correlation coefficients between chemical composition and *in vitro* degradation characteristics of crude protein of *T. esculentum* and sunflower seed cakes

	MBCD					WMBC				
	a	b	u	k_d	ED	a	b	u	k_d	ED
CP	0.131	0.699*	-0.575*	-0.768*	0.785*	0.382	0.573*	-0.574*	-0.123	0.466*
ADF	-0.437	-0.146	0.411	0.566*	-0.569*	-0.152	-0.430	0.366	0.540*	-0.493*
ADL	0.841*	-0.321	0.624*	0.271	-0.218	0.497*	-0.355	0.482*	0.538*	-0.594*
CEL	-0.466*	-0.075	0.253	0.591*	-0.497*	-0.241	-0.527*	0.242	0.542*	-0.044
HEMI	0.435	0.436	-0.060	-0.041	0.020	0.357	0.845*	-0.238	-0.748*	0.577*
CT	0.011	-0.566*	0.450	0.345	-0.151	0.200	-0.228	0.252	0.270	-0.149
	RMBC					WSSC				
	a	b	u	k_d	ED	a	b	u	k_d	ED
CP	0.104	0.441	-0.281	-0.245	0.312	-0.659*	0.494*	-0.526*	-0.376	0.501*

ADF	-0.258	-0.196	0.250	0.550*	-0.115	-0.363	-0.386	0.389	0.147	-0.267
ADL	0.126	-0.616*	0.400	0.729*	-0.521*	0.055	-0.018	0.023	0.266	-0.192
CEL	-0.017	-0.222	0.163	0.632*	-0.283	0.611*	-0.522*	0.544*	0.663*	-0.700*
HEMI	0.275	0.725*	-0.645*	-0.078	0.613*	0.400	0.31	-0.327	-0.235	0.323
CT	0.109	-0.646*	0.430	-0.023	-0.477*	0.720*	-0.701*	0.721*	-0.342	-0.782*

CP-crude protein; ADF – acid detergent fibre; ADL- acid detergent fibre; Cel – cellulose; Hemi- hemicellulose; CT- condensed tannin; MBCD – *Morama* bean cake decorticated; WMBC – whole *Morama* bean cake; RMBC – roasted *Morama* bean cake; WSSC-whole sunflower seed cake; *significant at $p<0.05$, *a*, *b*, and *u* represent soluble, slowly degradable, and undegradable fractions, respectively, *ED* - effective crude protein degradability at 0.02, 0.05, and 0.08/h ruminal passage rate

3.5.3.5 Model development from the *in vitro* DM degradation characteristics

Table 3.7 describes in detail how a model for predicting the soluble fractions (*a*) in the *in vitro* dry matter (DM) degradation of *T. esculentum* and sunflower seed cake was developed. Only the soluble fraction (*a*) of the DM of WSSC, WMBC, MBCD, and RMBC were highly predicted by the stepwise regression technique. By including DM and acid detergent fiber (ADF) in the equations, it was possible to account for 50%, 54%, and 84% of MBCD, WMBC, and RMBC, respectively. WSSC was removed from model development as its parameter contribution was not significant.

For the slowly degradable fraction (*b*), MBCD and RMBC had useful regression models. The DM *b* of MBCD included ADF and CT as the first and second predictors, while RMBC required DM, ADF, and ADL variables in the equation. MBCD and RMBC were the feed parameters used to generate prediction equations for the slowly degradable fractions. WMBC and WSSC were removed from model development as their parameter contribution was not significant.

DM, ADF, and CT were included in the regression model used to predict the nondegradable fraction (*u*) and were able to account for 81% and 92% of the variation for MBCD and RMBC,

respectively (Table 3.7). WMBC and WSSC were removed from model development because their parameter contributions were not significant.

Table 3.7: Equations for estimating *in vitro* degradation characteristics of dry matter of *T. esculentum* (MBCD, RMBC and WMBC) and sunflower seed cakes

MBCD	R ²	WMBC	R ²
$a = -111.76699 + 1.47191 \times \text{DM}$	0.502	$a = 29.83701 - 0.28492 \times \text{DM}$	0.543
$b = 48.56072 - 0.46683 \times \text{ADF} + 12.16352 \times \text{CT}$	0.750	$b = \text{No variable met the 0.1500 significance level}$	0.000
$u = 25.17699 + 0.57189 \times \text{ADF} - 13.76935 \times \text{CT}$	0.805	$u = \text{No variable met the 0.1500 significance level}$	0.000
$k_d = \text{No other variable met the 0.1500 significance level}$	0.000	$k_d = \text{No variable met the 0.1500 significance level}$	0.000
$\text{EDM}_2 = -244.26264 + 3.20225 \times \text{DM}$	0.765	$\text{EDM}_2 = \text{No variable met the 0.1500 significance level}$	0.000
$\text{EDM}_5 = \text{No variable met the 0.1500 significance level}$	0.00	$\text{EDM}_5 = \text{No variable met the 0.1500 significance level}$	0.00
$\text{EDDM}_8 = \text{No variable met the 0.1500 significance level}$	0.00	$\text{EDM}_8 = \text{No variable met the 0.1500 significance level}$	0.00
RMBC	R ²	WSSC	R ²
$a = -138.00352 + 1.20190 \times \text{DM} + 1.98591 \times \text{ADF}$	0.837	$a = \text{No variable met the 0.1500 significance level}$	0.000
$b = -154.83437 + 1.61283 \times \text{DM} + 2.13218 \times \text{ADF} - 1.66056 \times \text{ADL}$	0.929	$b = \text{No variable met the 0.1500 significance level}$	0.000
$u = 354.42587 - 2.48574 \times \text{DM} - 3.52054 \times \text{ADF}$	0.919	$u = \text{No variable met the 0.1500 significance level}$	0.000
$k_d = -0.21925 + 0.00285 \times \text{DM} + 0.00357 \times \text{ADF} + 0.00734 \times \text{ADL}$	0.927	$k_d = 0.35437 - 0.00252 \times \text{DM} + 0.02464 \times \text{ADL}$	0.851
$\text{EDM}_2 = \text{No other variable met the 0.1500 significance level}$	0.000	$\text{EDM}_2 = \text{No variable met the 0.1500 significance level}$	0.000
$\text{EDM}_5 = \text{No other variable met the 0.1500 significance level}$	0.00	$\text{EDM}_5 = \text{No variable met the 0.1500 significance level}$	0.00
$\text{EDM}_8 = \text{No other variable met the 0.1500 significance level}$	0.00	$\text{EDM}_8 = \text{No variable met the 0.1500 significance level}$	0.00

a , b , and u represent soluble, slowly degradable, and undegradable fractions, respectively ED - effective crude protein degradability at 0.02, 0.05, and 0.08/h ruminal passage rate, R^2 -coefficient of regression, MBCD – *Morama* bean cake decorticated; WMBC – whole *Morama* bean cake; RMBC – roasted *Morama* bean cake; WSSC-whole sunflower seed cake

The regression model accommodating DM, ADF, and ADL was able to predict the fractional degradation rate (k_d) and accounted for 93% and 85% for RMBC and WSSC, respectively. The model was successful for RMBC and WSSC but not for MBCD and WMBC due to insignificant parameter contributions.

The regression model for predicting effective degradability (ED) for feed was only feasible with decorticated *Morama* bean cake (MBCD), in which dry matter (DM) accounted for 76% of the ED variation. The other feeds (roasted *Morama* bean cake (RMBC), whole *Morama* bean cake (WMBC), and whole sunflower seed cake (WSSC) were excluded from model development due to insignificant parameter contributions.

3.5.3.6 Model developments of the *in vitro* CP degradation characteristics

The development of the prediction models for soluble fractions (a) for *in vitro* crude protein (CP) degradation of *T. esculentum* and sunflower seed cake are shown in Table 3.8. The models predicted that the soluble fractions for MBCD and WSSC would be 49% and 78%, respectively, which is explained by the use of CP (MBCD) and condensed tannins (CT) (WSSC) in the equations. RMBC and WMBC variables were excluded from model development due to insignificant parameter contributions.

Table 3.8: Equations for estimating *in vitro* degradation characteristics of crude protein of *T. esculentum* (MBCD, RMBC, and WMBC) and sunflower seed cakes

MBCD	R ²	WMBC	R ²
$a = 22.61250 - 0.51667 \times \text{CP}$	0.489	$a = \text{No variable met the 0.1500 significance level}$	0.000
$b = 91.76163 + 8.13043 \times \text{CT}$	0.321	$b = 20.70391 + 0.41437 \times \text{CP} + 7.51651 \times \text{CT}$	0.617
$u = -19.52500 + 0.63333 \times \text{CP}$	0.331	$u = 77.96161 - 0.60809 \times \text{CP} - 1.78099 \times \text{ADL} - 9.08016 \times \text{CT}$	0.648
$k_d = 0.94587 - 0.01983 \times \text{CP}$	0.589	$k_d = \text{No variable met the 0.1500 significance level}$	0.000
$\text{ED} = 130.22500 - 1.38333 \times \text{CP}$	0.616	$\text{ED} = 18.12819 + 0.30286 \times \text{CP} + 2.15639 \times \text{ADL}$	0.6358
RMBC	R ²	WSSC	R ²
$a = \text{No variable met the 0.1500 significance level}$	0.000	$a = 1.91433 + 0.19435 \times \text{CP} - 3.99449 \times \text{CT}$	0.783
$b = 28.20066 + 17.73128 \times \text{CT}$	0.418	$b = 94.75235 - 28.61079 \times \text{CT}$	0.501
$u = \text{No variable met the 0.1500 significance level}$	0.000	$u = -1.17405 + 33.36395 \times \text{CT}$	0.520
$k_d = 0.20117 + 0.05150 \times \text{ADL}$	0.532	$k_d = 0.38589 - 0.71871 \times \text{CT}$	0.496
$\text{ED} = \text{No other variable met the 0.1500 significance level}$	0.000	$\text{ED} = 92.80241 - 70.52813 \times \text{CT}$	0.612

a , b , and u represent soluble, slowly degradable, and undegradable fractions, respectively, ED - effective crude protein degradability at 0.02, 0.05, and 0.08/h ruminal passage rate, R^2 -coefficient of regression, MBCD – *Morama* bean cake decorticated; WMBC – whole *Morama* bean cake; RMBC – roasted *Morama* bean cake; WSSC-whole sunflower seed cake

The regression models used to predict slowly degrading fractions in whole *Morama* bean cake (WMBC) and whole sunflower seed cake (WSSC) accounted for 62% and 50%, respectively, which is explained by the use of crude protein (CP) and condensed tannins (CT) in the equations. The roasted *Morama* bean cake (RMBC) did not have a significant nondegradable fraction (U) and was removed from model development.

The regression models used to predict degradation rates accounted for 59%, 53%, and 50% in decorticated *Morama* bean cake (MBCD), RMBC, and WSSC, respectively, which is explained by the use of CP, acid detergent lignin (ADL), and CT variables in the equations. The whole *Morama* bean cake (WMBC) did not show significant degradation rates and was removed from model development.

The regression models predicting mean effective degradability (ED) for decorticated *Morama* bean cake (MBCD), whole *Morama* bean cake (WMBC), and whole sunflower seed cake (WSSC) registered for 62%, 64%, and 61%, respectively, which is explained by the use of crude protein (CP), acid detergent lignin (ADL), and condensed tannins (CT) in the equations. The roasted *Morama* bean cake (RMBC) did not exhibit a viable model to predict ED and was removed from model development.

3.5.4 *In Situ* Rumen Degradability

3.5.4.1 Dry matter and Crude protein disappearance of sunflower and *Morama* oilseed cakes

The results of *in situ* dry matter (DM) and crude protein (CP) disappearance of test feeds (sunflower and *Morama* cake) at each time point are shown in Figures 3.3 and 3.4. For each of the diets tested, it took up to 48 hours for the component to disappear. Among feeds, roasted *Morama* bean cake (RMBC) had the highest (36.5%; $P < 0.05$) DM disappearance at zero hours, while whole *Morama* bean cake (WMBC) had the lowest value (18.2%; $P < 0.05$). Roasted *Morama* bean cake and decorticated *Morama* bean cake (MBCD) did not differ (93.3% vs. 89.4%; $P > 0.05$) in disappearance of DM after 48 hours, while whole sunflower seed cake (WSSC) and whole *Morama* bean cake (WMBC) had the lowest disappearance (63.9% vs. 57.3%; $P < 0.05$) at 48

hours. Therefore, the highest DM disappearances feed parameters at 48 hours were, in descending order, as follows: RMBC, MBCD, WSSC, and WMBC (Figure 4.1; $P < 0.05$).

When crude protein (CP) disappeared, the more rapidly soluble fractions were higher for whole sunflower seed cake (WSSC) and roasted *Morama* bean cake (RMBC) (35% and 38.1%, respectively; $P < 0.05$), while they were lower for decorticated *Morama* bean cake (MBCD) and whole *Morama* bean cake (WMBC) (20% and 17%, respectively). CP disappearance at 24 hours was higher in WSSC and RMBC (84.1% and 82.3%, respectively; $P < 0.05$). The lowest CP disappearance value at 24 hours was for WMBC (44%; $P < 0.05$). WSSC and RMBC had even greater disappearance of CP after 48 hours (95.8% and 97.7%, respectively; Figure 3.4).

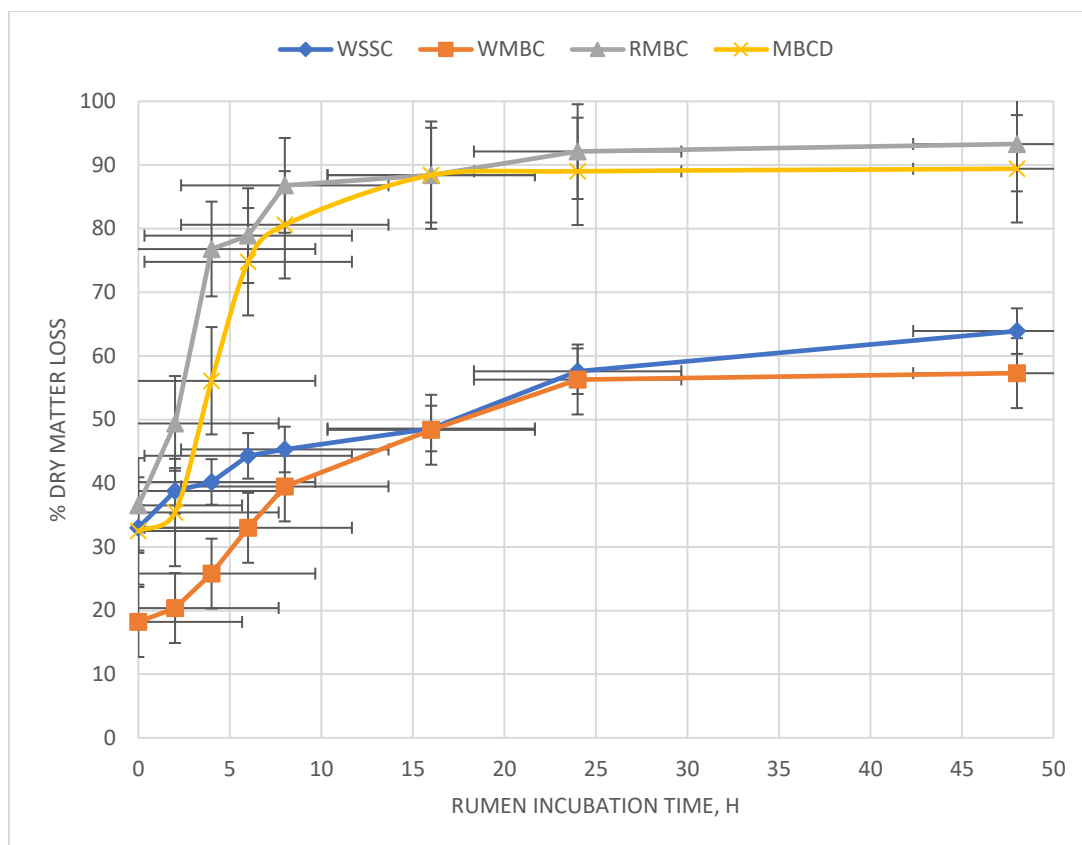


Figure 3.3: *In situ* dry matter (%) losses fitted degradation curves of WSSC: whole sunflower seed cake; RMBC: roasted *Morama* bean cake; MBCD: *Morama* bean cake decorticated; WMBC: whole *Morama* bean cake; + Error bars indicate standard deviations

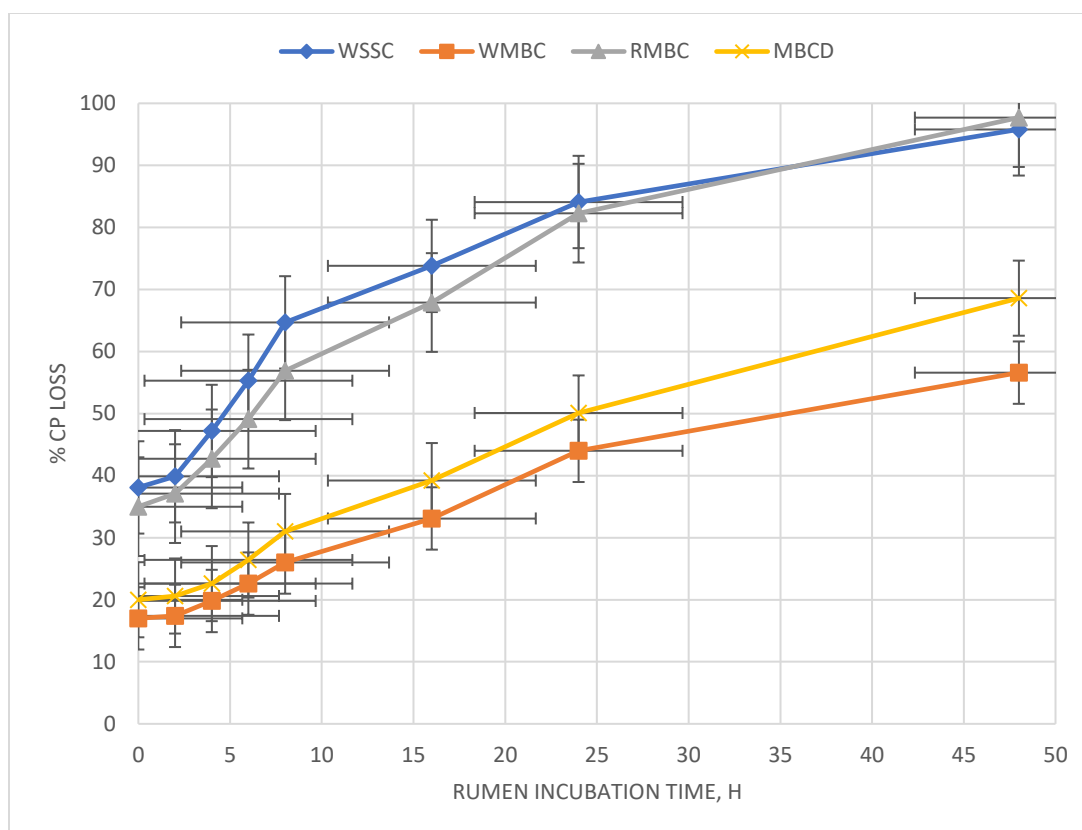


Figure 3.4: *In situ* crude protein (%) losses fitted degradation curves of WSSC: whole sunflower seed cake; RMBC: roasted *Morama* bean cake; MBCD: *Morama* bean cake decorticated; WMBC: whole *Morama* bean cake; + Error bars indicate standard deviations.

3.5.4.2 Dry matter and crude protein degradation kinetics

The percentage of the rapidly soluble (a) and slowly but rumen degradable (b) dry matter (DM) and crude protein (CP) fractions, as well as the remaining non-degradable pools (u), the fractional degradation rates (k_d/h), and the effective degradability (ED) from various oilseed feeds, are listed in Table 3.9. The values of a , b , u , k_d , and ED parameters of DM varied depending on the oilseed press cake. The highest values for a (37.5%), b (44.6%), k_d (0.02/h), and effective dry matter degradation (EDDM) at different rumen drainage rates were from roasted *Morama* bean cake (RMBC). The lowest ($P < 0.05$) a , b , k_d , and ED values for DM were in the decorticated *Morama*

bean cake (MBCD). MBCD had the highest non-degradable dry matter content (42.5%) among oilseed cakes.

The a , b , and k_d parameter values for crude protein (CP) ranged from 6.3% (whole *Morama* bean cake (WMBC)) to 18.3% (roasted *Morama* bean cake (RMBC), 15.3% (WMBC) to 53% (RMBC), and 0.035 (RMBC) to 0.062 /h, respectively. The effective degradability (ED) of CP at 0.02, 0.05, and 0.08 efflux rates ranged from 15.1% (WMBC) to 45.4% (RMBC), 10.4% (WMBC) to 29.4% (RMBC), and 7.9% (WMBC) to 22.6% (MBCD).

Table 3.9: *In situ* averages for estimates of kinetic degradation of dry matter (DM) and crude protein (CP) of sunflower and *Morama* cakes, defined by soluble fraction (a , %), slowly degradable fraction (b , %), non-degradable residual amounts (u , % = 100-a-b), fractional disappearance rate (k_d , %/h) and effective degradability (ED, %) which assumes outflow rates (kp, %/h)

Parameter	WSSC	WMBC	RMBC	MBCD	SE
<i>Disappearing fraction, a (%)</i>					
DM	34.8 ^b	19.7 ^c	37.5 ^a	13.4 ^d	0.62*
CP	13 ^b	6.3 ^c	18.3 ^a	16.7 ^a	0.37*
<i>Slowly disappearing fraction, b (%)</i>					
DM	43.9	44.2	44.6	44.1	0.94 ^{ns}
CP	25.4 ^c	15.3 ^d	53 ^a	35 ^b	0.53*
<i>Undegradable residual pool (%)</i>					
DM	21.3 ^c	36.1 ^b	17.9 ^c	42.5 ^a	1.08*
CP	61.6 ^b	78.4 ^a	28.7 ^d	48.3 ^c	2.36*
<i>Rate of degradation of b fraction, k_d (%/h)</i>					
DM	0.34 ^b	0.11 ^c	2.02 ^a	0.15 ^c	0.01*
CP	0.062 ^a	0.046 ^b	0.035 ^c	0.062 ^a	0.05*
<i>Ruminal availabilities¹, ED = 0.02/h</i>					
DM	74.3 ^b	54.1 ^c	81.3 ^a	50.7 ^c	0.41*
CP	29 ^c	15.1 ^d	45.4 ^a	39.1 ^b	0.31*
<i>Ruminal availabilities², ED = 0.05/h</i>					
DM	68.6 ^b	43.9 ^c	80.1 ^a	43.1 ^c	0.32*
CP	21.3 ^b	10.4 ^c	29.4 ^a	28.6 ^a	0.21*
<i>Ruminal availabilities³, ED = 0.08/h</i>					
DM	63.7 ^b	37 ^c	79 ^a	37.5 ^c	0.44*

CP	16.8 ^b	7.9 ^c	21.7 ^a	22.6 ^a	0.33*
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^{1,2,3} ED = $a + (b * k_d)/(k_d + kp)$; ^{1,2,3} Kp – assumed rumen outflow rate (0.02, 0.05, & 0.08/h)

^{a, b, c, d} means in the same raw with different superscripts differ ($P < 0.05$); * $P < 0.05$ and ns, not significant

WSSC – whole sunflower seed cake; WMBC- whole morama bean cake; RMBC- roasted morama bean cake; MBCD – morama bean cake decorticated

3.5.4.3 Relationship between dry matter degradation kinetics and chemical composition

The correlation coefficients (r) between the dry matter (DM) degradation fractions of the oilseed cakes and the other chemical components are given in Table 3.9. The dry matter degradation fractions (DMDa, b , and u) of decorticated *Morama* bean cake (MBCD) were positively correlated with the organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent lignin (ADL), condensed tannins (CT), and non-fiber carbohydrates (NFC) contents. Thus, a high positive correlation was found between OM ($r = 0.70$), CP ($r = 0.70$), and CT ($r = 0.91$), and a moderate correlation ($r = 0.68$) between CP and b was found; while for NDF and ADL, a correlation with u was $r = 0.66$ and $r = 0.56$, respectively ($P < 0.05$).

In roasted *Morama* bean cake (RMBC), there was a correlation between CP and a ($r = 0.72$; $P < 0.05$) and between OM and a ($r = 0.56$; $P < 0.05$). OM was also associated with effective degradability at ED2 ($r = 0.52$), ED5 ($r = 0.53$), and ED8 ($r = 0.53$). There was also an average association of CP with k_d ($r = 0.55$) and a moderate association with ED2 and ED8 ($r = 0.65$ and $r = 0.67$, respectively). Both CT and NFC showed a negative association with k_d ($r = -0.53$ and $r = -0.60$, respectively).

Whole sunflower seed cake (WSSC) showed a negative correlation of organic matter (OM) with the fractional degradation rate (k_d) and the non-degradable fraction (u) ($r = -0.52$ and $r = -0.93$, respectively). Meanwhile, the crude protein (CP) of the same feed ingredient was positively related

to a , b , k_d , and effective degradability values (ED2, ED5, and ED8) ($r = 0.61$, $r = 0.52$, $r = 0.72$, $r = 0.73$, $r = 0.77$, and $r = 0.80$, respectively; $P < 0.05$). Acid detergent fiber (ADF) was negatively correlated with the rapidly soluble fraction (a) ($r = -0.75$), resulting in negative effective degradability at all levels (ED2, ED5, and ED8) ($r = -0.65$, $r = -0.63$, and $r = -0.63$). Conversely, acid detergent lignin (ADL) was positively correlated with the rapidly soluble fraction ($r = 0.67$) at all levels of effective degradability (ED2, ED5, and ED8), represented by the values $r = 0.60$, $r = 0.56$, and $r = 0.58$, respectively. Condensed tannins (CT) were negatively associated with the non-degradable fraction and the fractional degradation rate ($r = -0.78$ and $r = -0.63$, respectively), except for the insoluble but rumen-degradable fraction (b ; $r = 0.59$), where it showed a positive correlation. Although non-fiber carbohydrates (NFC) were positively associated with the b -fraction ($r = 0.58$), all effective degradability levels (ED2, ED5, and ED8) were negatively correlated with it ($r = -0.59$, $r = -0.58$, and $r = -0.61$).

As for WMBC, CP was positively correlated (although slightly lower) with all degradation kinetics (a , b , u , and k_d), including all levels of effective degradation (ED2, ED5, and ED8), at equal rates ($r = 0.47$). ADF was positively associated with the rapidly soluble fraction ($r = 0.64$) but negatively associated with the non-degradable fraction ($r = -0.58$). Regardless, CT showed a positive association with effective degradation ($r = 0.50$) at assumed rumen passage rate of 0.02/h.

Table 3.10: Correlation coefficients between chemical composition (% or g/kg) and in situ dry matter degradation kinetic fractions (%) of sunflower and *Morama* oilseed press cakes

Morama bean cake decorticated (MBCD)								Roasted morama bean cake (RMBC)							
Para	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	<i>ED2</i>	<i>ED5</i>	<i>ED8</i>	Para.	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	<i>ED2</i>	<i>ED5</i>	<i>ED8</i>
OM	0.70*	-0.44	-0.43	-0.04	-0.31	-0.32	-0.32	OM	0.56*	0.43	0.43	0.32	0.52*	0.53*	0.53*
CP	0.70*	0.68*	0.3	-0.4	-0.54*	-	-0.52*	CP	0.72*	0.49	-0.32	0.55*	0.65*	0.45	0.67*
						0.50*									
NDF	0.13	-0.42	0.66*	-0.03	-0.29	-0.22	-0.21	NDF	-0.27	-0.39	-0.41	0.03	-0.35	-0.31	-0.34
ADF	0.73*	-0.33	-0.12	-0.14	-0.16	-0.15	-0.15	ADF	-0.22	0.07	0.21	-0.07	-0.09	-0.14	-0.08
ADL	0.23	-0.23	0.56*	0.09	-0.11	-0.06	-0.44	ADL	0.03	-0.01	-0.34	0.31	0.01	0.16	0.12
CT	0.91*	-0.09	-0.32	0.09	0.09	0.09	0.08	CT	-0.04	0.34	-0.44	-0.53	0.15	0.33	0.13
NFC	-0.43	0.62*	-0.64*	0.27	0.47	0.42	0.4	NFC	-0.45	-0.13	0.42	-0.60*	-0.32	-0.15	-0.34
Whole morama bean cake (WMBC)								Whole sunflower seedcake (WSSC)							
Para	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	<i>ED2</i>	<i>ED5</i>	<i>ED8</i>	Para.	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	<i>ED2</i>	<i>ED5</i>	<i>ED8</i>
OM	0.44	0.44	0.44	0.44	0.44	0.44	0.44	OM	0.03	0.06	-0.52*	-0.93*	0.33	0.52*	0.52
CP	0.47	0.47	0.47	0.47	0.47	0.47	0.47	CP	0.61*	0.52*	-0.37	0.72*	0.73*	0.77*	0.80*
NDF	0.25	0.25	0.25	0.25	0.25	0.25	0.25	NDF	0.27	0	-0.3	-0.19	0.2	0.13	0.12
ADF								ADF	-0.75*	-0.12	-0.38	-0.21	-0.65*	-0.63*	-0.63*
	0.64	0.02	-0.58	0.17	0.51	0.12	0.59*								
ADL	-0.01	0.22	-0.3	-0.23	0.18	0.19	0.11	ADL	0.67*	0.16	-0.11	0.26	0.60*	0.56*	0.58*
CT	0.16	-0.12	0.13	-0.08	-0.03	0.50	-0.05	CT	-0.05	0.59*	-0.78*	-0.63*	-0.33	-0.38	-0.38
NFC	-0.36	0.13	0.36	0.15	-0.12	-0.18	-0.07	NFC	-0.43	0.58*	0.42	-0.41	-0.59*	-0.58*	-0.61*

Para. refers to parameters , *a*: Soluble fraction (%),*b*: Slowly degradable fraction (%),*u*: Non-degradable residual amounts (%),*k_d*: Fractional disappearance rate (per hour), *ED2*, *ED5*, *ED8*: Effective degradability at outflow rates of 2%, 5%, and 8% per hour, respectively, *indicates significant correlation at $p < 0.05$

3.5.4.4 Relationship between crude protein degradation kinetic fractions and chemical composition

The correlation coefficients (r) between the degradation kinetics of the crude protein and the chemical composition are given in Table 3.11. The crude protein of MBGD was negatively correlated with the non-degradable fraction ($r = -0.64$) and the fractional degradation rate ($r = -0.51$), except for the b ($r = 0.59$). NDF correlated positively with the rapidly soluble fraction ($r = 0.84$) and negatively with the non-degradable fraction ($r = -0.57$), while ADF correlated with the b ($r = 0.64$) and also negatively with the u ($r = -0.51$). ADL was only negatively correlated with u ($r = -0.72$), while NFC was negatively correlated with a ($r = -0.71$) and positively correlated with u ($r = 0.74$). The feed component also tended to show a negative relationship between CT and effective degradability ED₂, ED₅, and ED₈) ($r = -0.85$, $r = -0.90$ and $r = -0.91$, respectively; $P < 0.05$).

For roasted *Morama* bean cake (RMBC), there was a moderate correlation between crude protein (CP) and the a ($r = 0.58$; $P < 0.05$), and between neutral detergent fibre (NDF) and the a ($r = 0.51$; $P < 0.05$). Non-fibre carbohydrates (NFC) were moderately correlated with the b ($r = 0.66$; $P < 0.05$). The b ($r = -0.50$; $P < 0.05$) correlated negatively with the acid detergent lignin (ADL) content. Another negative and moderate correlation observed was between the rate of digestion (k_d) and condensed tannins (CT) ($r = -0.53$; $P < 0.05$).

The degradation constants of crude protein (CP) from WMBC showed a negative and moderate correlation between its CP solubility and fractional degradation rate ($r = -0.54$; $P < 0.05$). There was a negative relationship between non-fiber carbohydrates (NFC) and the non-degradable

fraction (u ; $r = -0.50$; $P < 0.05$). Condensed tannin (CT) was also negatively correlated with the slowly degradable fraction (b ; $r = -0.58$; $P < 0.05$).

The degradation properties of crude protein (CP) from WSSC showed positive, moderate to strong correlations with organic matter and the rapidly dissolving fraction (a ; $r = 0.69$) and the slow degrading fraction (b ; $r = 0.74$). There was a negative correlation with the non-degradable fraction (u ; $r = -0.76$) and a moderate correlation with the effective organic matter degradability at a flow rate of 0.02/h ($r = 0.57$). CP correlated moderately with the rapidly soluble fraction ($r = 0.51$), strongly with the slowly degradable fraction ($r = 0.78$), negatively and strongly with the non-degradable fraction ($r = -0.74$), and was strongly associated with the effective CP degradability across all levels (ED2, ED5, and ED8) ($r = 0.84$, $r = 0.86$, and $r = 0.86$, respectively; $P < 0.05$). Acid detergent lignin (ADL) had a moderate association with the fractional degradation rate (k_d ; $r = 0.56$), while non-fiber carbohydrates (NFC) had a negative association with the same fraction ($r = -0.53$). Condensed tannin (CT) of WSSC feed ingredient showed negative correlations with a , b , u , k_d , and effective CP degradability ($r = -0.62$, $r = -0.86$, $r = -0.83$, $r = -0.56$, and $r = -0.68$; $P < 0.05$).

Table 3.11: Correlation coefficients between chemical composition (% , g/kg) and *in situ* crude protein degradation kinetic fractions (%) of sunflower (WSSC) and *Morama* (MBCD, RMBC, and WMBC) oilseed press cakes

<i>Morama</i> bean cake decorticated (MBCD)								Roasted <i>Morama</i> bean cake (RMBC)							
Para	<i>a</i>	<i>B</i>	<i>u</i>	<i>k_d</i>	<i>ED2</i>	<i>ED5</i>	<i>ED8</i>	Para	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	<i>ED2</i>	<i>ED5</i>	<i>ED8</i>
OM	0.36	0.46	-0.41	-0.35	-0.23	-0.33	0.48	OM	-0.16	-0.10	0.15	0.18	0.06	0.13	0.13
CP	0.44	0.59*	-	-	-0.30	-0.42	-0.45	CP	0.51*	0.07	-0.29	-0.24	-0.30	-0.29	-0.28
NDF	0.84*	0.26	0.64*	0.51*	0.02	0.32	0.2	NDF	0.58*	0.20	-0.41	-0.33	-0.19	-0.29	-0.28
ADF	0.45	0.64*	-	-	-0.37	-0.17	0.28	ADF	0.35	0.47	-0.49	0.55*	0.62*	0.57*	0.56*
ADL	0.48	0.22	0.51*	-	-0.17	0.16	-0.01	ADL	-0.37	-	0.52*	-0.49	-	- 0.52*	- 0.51*
NFC	-	-0.43	0.72*	0.74*	0.24	-0.05	0.08	NFC	0.36	0.50*	-	-0.15	-0.15	0.18	-0.18
CT	0.71*	-0.08	-0.36	-	-	-	-	CT	-0.04	0.66*	0.62*	-	0.15	0.33	0.13
				0.91*	0.85*	0.90*	0.91*			0.34	-0.44	0.53*			
Whole <i>Morama</i> bean cake (WMBC)								Whole sunflower seed cake (WSSC)							
Para	<i>a</i>	<i>B</i>	<i>u</i>	<i>k_d</i>	<i>ED2</i>	<i>ED5</i>	<i>ED8</i>	Para	<i>a</i>	<i>b</i>	<i>u</i>	<i>k_d</i>	<i>ED2</i>	<i>ED5</i>	<i>ED8</i>
OM	-0.13	-0.31	0.22	0.21	-0.15	-0.03	-0.04	OM	0.69*	0.74*	-	0.34	0.57*	0.49	0.48
CP	0.47	-0.07	-0.32	-	0.01	-0.20	-0.26	CP	0.51*	0.78*	0.76*	0.84*	0.84*	0.86*	0.86*
NDF	0.28	0.25	-0.31	0.54*	0.19	0.12	0.07	NDF	-0.12	-0.15	-	0.02	-0.13	-0.06	-0.06
ADF	0.22	-0.34	-0.02	0.06	0.08	0.09	0.09	ADF	-0.11	0.32	0.74*	0.34	0.32	0.32	0.32
ADL	-0.23	-0.17	0.22	0.03	-0.24	-0.24	-0.21	ADL	-0.03	0.21	0.15	0.56*	0.36	0.43	0.45
NFC	-0.58*	-0.19	0.50*	0.33	-0.34	-0.21	-0.13	NFC	0.01	-0.39	0.28	-	-0.42	-0.46	-0.47
CT	0.00	-	0.24	0.39	-0.01	0.16	0.19	CT	-	-	0.83*	0.53*	-	- 0.69*	- 0.68*
		0.58*							0.62*	0.86*		0.56*	0.74*		

Para. refers to parameters, *a*: Soluble fraction (%), *b*: Slowly degradable fraction (%), *u*: non-degradable residual amounts (%), *k_d*: Fractional

disappearance rate (per hour), ED2, ED5, ED8: Effective degradability at outflow rates of 2%, 5%, and 8% per hour, respectively, *indicates significant correlation at $p < 0.05$.

3.5.4.5 Modelling of *in situ* DM degradation kinetic fractions and the chemical composition

Prediction equations describing the relationships between degradation parameters of DM and chemical composition of sunflower and *Morama* oilseed cakes (MBCD, RMBC, and WMBC) are presented in Table 3.12. According to the regression coefficient of determination (R^2), the DM soluble fraction (a) for MBCD was best described by CT + ADF ($R^2 = 0.968$), while CP ($R^2 = 0.524$) accounted for RMBC. ADF ($R^2 = 0.405$) and ADF ($R^2 = 0.565$) were found to better predictors for WMBC and WSSC, respectively. The single predictor (ADF) of WMBC was not significant ($R^2 = 0.405$; $P > 0.05$).

The parameter b for MBCD and RMBC was predicted by crude protein (CP) ($R^2 = 0.464$ and $R^2 = 0.443$, respectively; $P < 0.05$). For WSSC and WMBC, the predictors were organic matter (OM) and condensed tannin (CT) ($R^2 = 0.360$ and $R^2 = 0.437$, respectively; $P > 0.05$), but these predictions were not significant.

The best predictor of the non-degradable parameter (u) was CT ($R^2 = 0.611$; $P < 0.05$) in the WSSC, while the same parameter (u) was marginally predicted by the RMBC ($R^2 = 0.339$; $P > 0.05$). The non-degradability parameters were predicted by NDF ($R^2 = 0.434$; $P > 0.05$) and ADF ($R^2 = 0.341$; $P > 0.05$) for MBCD and WMBC, respectively.

Parameter k_d was strongly predicted by OM + ADF ($R^2 = 0.963$; $P < 0.05$) for whole sunflower seedcake, but weakly ($P > 0.05$) by NFC in all other feed ingredients (MBCD, RMBC, and WMBC) as sole predictors (Table 3.11).

The effective dry matter degradability (EDDM) benefited from three parameters (organic matter (OM), crude protein (CP), and condensed tannin (CT)) for roasted *Morama* bean cake (RMBC) at different rumen effluent rates (0.02, 0.05, and 0.08/h) and showed significant and strong predictions ($R^2 = 0.890$, $R^2 = 0.923$, and $R^2 = 0.900$; $P < 0.05$). The EDDM of whole sunflower seed cake showed moderate model prediction as indicated by a single predictor, CP ($R^2 = 0.535$, $R^2 = 0.594$, and $R^2 = 0.644$) at different rumen discharge rates (0.02, 0.05, and 0.08/h, respectively). However, EDDM was marginally predictive only for MBCD and WMBC, and both were not significant (Table 3.11; $P > 0.05$).

Table 3.12: *In situ* regression equations showing the relationship between the rumen degradation parameters of DM(%) and the chemical composition (% or g/kg) of sunflower and *Morama* oilseed presscakes

Morama bean cake decorticated (MBCD)	R²	P-value
$a = 3.972 + 6.039 \text{ CT} + 6.039 \text{ ADF}$	0.968	0.0005
$b = 98.52 - 1.700 \text{ CP}$	0.464	0.063
$u = -8.387 + 2.009 \text{ NDF}$	0.434	0.076
$k_d = 2.314 - 0.015 \text{ NFC}$	0.279	0.126
$ED2 = -63.05 + 0.118 \text{ OM} - 1.008 \text{ CP} - 7.125 \text{ CT}$	0.178	0.147
$ED5 = -57.31 + 0.017 \text{ OM} - 1.140 \text{ CP} - 7.046 \text{ CT}$	0.119	0.142
$ED8 = -46.53 + 0.171 \text{ OM} - 1.250 \text{ CP} - 6.005 \text{ CT}$	0.231	0.116
Roasted Morama bean cake (RMBC)	R²	P-value
$a = 11.947 + 0.627 \text{ CP}$	0.524	0.042
$b = 93.73 - 1.674 \text{ CP}$	0.443	0.076
$u = -83.67 + 4.011 \text{ CT}$	0.339	0.184
$k_d = 2.410 - 0.023 \text{ NFC}$	0.358	0.117
$ED2 = -71.046 + 1.019 \text{ OM} + 1.169 \text{ CP} + 86.559 \text{ CT}$	0.890	0.017
$ED5 = -76.18 + 0.8671 \text{ OM} + 1.159 \text{ CP} + 75.67 \text{ CT}$	0.923	0.013
$ED8 = -69.28 + 0.9770 \text{ OM} + 1.173 \text{ CP} + 83.65 \text{ CT}$	0.900	0.016
Whole Morama bean cake (WMBC)	R²	P-value
$a = -15.43 + 3.115 \text{ ADF}$	0.405	0.090

$b = 83.16 - 1.564 \text{ CT}$	0.437	0.156
$u = 94.49 - 5.118 \text{ ADF}$	0.341	0.129
$k_d = 2.346 - 0.012 \text{ NFC}$	0.445	0.108
$ED_2 = -84.66 + 0.016 \text{ OM} + 1.114 \text{ CP} + 75.34 \text{ CT}$	0.356	0.130
$ED_5 = 0.567 + 1.673 \text{ OM} - 2.006 \text{ CP}$	0.395	0.156
$ED_8 = 9.45451 + 2.389 \text{ ADF}$	0.349	0.123
Whole sunflower seedcake (WSSC)	R²	P-value
$a = 33.856 - 0.0278 \text{ ADF}$	0.565	0.031
$b = 14.526 + 0.3195 \text{ OM}$	0.360	0.116
$u = 20.936 + 9.2005 \text{ CT}$	0.611	0.022
$k_d = -0.631 + 0.0107 \text{ OM} - 0.0001 \text{ ADF}$	0.963	0.017
$ED_2 = 28.414 + 2.0017 \text{ CP}$	0.535	0.039
$ED_5 = 20.560 + 2.103 \text{ CP}$	0.594	0.025
$ED_8 = 10.963 + 2.319 \text{ CP}$	0.644	0.017
OM – organic matter; CP – crude protein; NDF – neutral detergent fibre; acid detergent fibre; ADL – acid detergent lignin; CT – condensed tannin; NFC – non fibre carbohydrate; a – rapid soluble fraction; b – degradable insoluble; u – undegradable residual pool; k_d – degradation rate; ED_2 – effective degradability at 2%; ED_5 – effective degradability at 5%; ED_8 – effective degradability at 8%; R^2 = coefficient of determination		

3.5.4.6 Modelling of *in situ* CP degradation kinetics and the chemical composition

The prediction equations describing the relationships between crude protein (CP) degradation parameters and the chemical composition of sunflower and *Moranga* oilseed cakes are presented in Table 3.13. The rapidly soluble fraction (*a*) of MBCD was best predicted by including neutral detergent fibre (NDF) in the equation ($R^2 = 0.703$; $P < 0.05$), while including non-fibre carbohydrates (NFC) from WMBC as a single predictor gave a lower value ($R^2 = 0.335$). Furthermore, a combination of NDF, NFC, and condensed tannin (CT) for RMBC predicted the 'a' fraction with an R^2 value of 0.623, while a combination of organic matter (OM) and acid detergent fibre (ADF) moderately predicted the fast-dissolving fraction ($R^2 = 0.680$; $P < 0.05$) of WSSC.

The best predictors of *b*-fraction of WMBC were combinations of CT and ADL ($R^2 = 0.762$) while it was CT and CP ($R^2 = 0.922$) for WSSC. ADF ($R^2 = 0.407$) and NFC ($R^2 = 0.431$) were found to have low determination coefficients for the same parameter for MBCD and RMBC, respectively. The non-degradable fraction of feed ingredients was predicted by a combination of NFC + CP, NFC - NDF - OM + CP and CT- CP, with coefficients ranging from $R^2 = 0.555$ (MBCD) to $R^2 = 0.847$ (WSSC). Fractional degradation rates (k_d) were moderately predicted by CT ($R^2 = 0.551$) for WMBC and a combination of ADF + ADL ($R^2 = 0.671$) for RMBC.

On the other hand, higher predictions for k_d were presented by CT ($R^2 = 0.824$) and CP ($R^2 = 0.707$) as single predictors for MBCD and WSSC, respectively. The effective degradability of CP (EDCP2, EDCP5, and EDC8) showed the highest ($P < 0.05$) R^2 values, ranging from 0.75 (RMBC) to 0.846 (WSSC), except for WMBC, which had the lowest R^2 values, ranging from 0.411 to 0.441 at different rumen outflow rates.

Table 3.13: *In-situ* regression equations showing the relationship between CP rumen degradation parameters and the chemical composition of sunflower (WSSC) and *Morama* (MBCD, RMBC and WMBC) oilseed press cake

Morama bean cake decorticated (MBCD)	R ²	P-value	Roasted morama bean cake (RMBC)	R ²	P-value
$a = -16.51 + 1.276 \text{ NDF}$	0.703	0.009	$a = -23.78 + 1.421 \text{ NDF} + 0.135 \text{ NFC} - 29.65 \text{ CT}$	0.623	0.051
$b = 4.381 + 1.381 \text{ ADF}$	0.407	0.189	$b = 49.08 + 0.154 \text{ NFC}$	0.431	0.077
$u = -164.80 + 6.117 \text{ NFC}$	0.555	0.034	$u = 311.7 - 0.355 \text{ NFC} - 4.676 \text{ NDF} - 1.852 \text{ OM} + 0.825 \text{ CP}$	0.748	0.027
$k_d = 1.264 - 3.486 \text{ CT}$	0.824	0.002	$k_d = 2.672 - 4.468 \text{ ADF} - 0.2591 \text{ ADL}$	0.671	0.067
$ED2 = 110.2 - 89.57 \text{ CT} - 1.252 \text{ NFC}$	0.841	0.013	$ED2 = 423.6 + 3.577 \text{ ADF} - 0.9219 \text{ NFC} - 11.37 \text{ NDF} - 4.126 \text{ OM} - 10.37 \text{ ADL}$	0.782	0.018
$ED5 = 74.47 - 139.0 \text{ CT}$	0.805	0.003	$ED5 = 916.7 + 8.256 \text{ ADF} - 1.547 \text{ NFC} - 17.49 \text{ NDF} - 4.949 \text{ OM} - 8.078 \text{ ADL}$	0.766	0.068
$ED8 = 78.0 - 166.1 \text{ CT}$	0.825	0.002	$ED8 = 748.4 + 12.39 \text{ ADF} - 1.840 \text{ NFC} - 18.15 \text{ NDF}$	0.704	0.006
Whole morama bean cake (WMBC)	R ²	P-value	Whole sunflower seed cake (WSSC)	R ²	P-value
$a = 26.9 - 0.492 \text{ NFC}$	0.335	0.133	$a = -32.82 + 0.598 \text{ OM} - 0.855 \text{ ADF}$	0.680	0.031
$b = 27.13062 - 17.2 \text{ CT} - 1.833 \text{ ADL}$	0.762	0.031	$b = -7.721 - 10.71 \text{ CT} + 1.534 \text{ CP}$	0.922	0.090
$u = -115 + 2.554 \text{ NFC} + 0.075 \text{ CP}$	0.562	0.012	$u = 103.2 + 14.20 \text{ CT} - 1.922 \text{ CP}$	0.847	0.073
$k_d = -23.46 - 3.447 \text{ CP} - 1.041 \text{ CT}$	0.551	0.034	$k_d = -0.307 + 0.016 \text{ CP}$	0.707	0.009
$ED2 = -112.3 - 1.117 \text{ ADF} + 0.034 \text{ CT} + 1.671 \text{ CP}$	0.411	0.120	$ED2 = -51.52 + 3.624 \text{ CP} - 13.77 \text{ CT}$	0.861	0.064
$ED5 = -115.0 - 0.0674 \text{ ADF} + 0.1560 \text{ CT} + 1.444 \text{ CP}$	0.432	0.132	$ED5 = 31.42 + 4.764 \text{ CP} - 17.91 \text{ CT} - 1.273 \text{ OM}$	0.937	0.072
$ED8 = -14.2 - 1.0071 \text{ ADF} + 1.312 \text{ CT} + 0.9671 \text{ CP}$	0.441	0.116	$ED8 = -74.90 + 4.031 \text{ CP}$	0.739	0.006

a – rapidly soluble fraction; b – insoluble but rumen degradable fraction; u – nondegradable fraction; k_d – fractional degradation rate; ED_2 – effective degradation at ruminal passage rate of 0.02/h;

3.5.5 *In vitro* versus *in-situ* rumen dry matter and crude protein degradability

The differences in the estimated kinetic degradability fractions (a , b , u and k_d), including the effective DM degradability and/or the effective CP degradability, obtained by *in vitro* Daisy^{II} and *in situ* techniques (respectively from Tables 3.4 and 3.9) are summarized in Table 3.14. The results of *in vitro* technique showed the lowest kinetic degradation rates of dry matter (DM) in all feeds, except for rapidly soluble fraction (a), fractional degradation rate (k_d) of MBCD, nondegradable fraction (u) of RMBC, nondegradable (u) and fractional degradation rate (k_d) of WMBC and only nondegradable fraction (u) of WSSC compared to *in situ* rumen technique (Table 3.14). In general, the calculated effective DM degradation quotient at passage rates of 0.02, 0.05 and 0.08/h for feed ingredients showed that RMBC and WSSC had the lowest degradation quotients than the other feeds.

Regardless of similar rumen passage rates, differences were found between *in vitro* and *in-situ* CP degradation in roasted *Morama* bean cake (RMBC). This was observed in the rapidly soluble fraction (a), the insoluble but rumen-degradable fraction (b), and the fractional degradation rate (k_d), which were underestimated by up to 39.9% by the *in vitro* Daisy^{II} incubator ($P < 0.05$). The effective CP degradation at passage rates of 0.02, 0.05 and 0.08/h for the same feed increased by a maximum of 26.9% points in the *in vitro* Daisy^{II} incubator ($P < 0.05$) where the underestimation was indicated by a negative quotient (Table 3.14).

Table 3.14: Comparison of estimates of degradation kinetics (a [%], b [%], u [%], and k_d [h^{-1}]) of *in vitro* Daisy^{II} (24 h incubation time) and *in situ* (48 h incubation time) and effective DM [%] and CP [%] degradation at 0.02, 0.05, and 0.08/h assumed ruminal passage rate

Dry matter							
Feed ingredient	$a\%$	$b\%$	$u\%$	$k_d\%/h$	ED _{2/h}	ED _{5/h}	ED _{8/h}
Moroma bean cake decorticated	0.813	-0.086	-0.165	0.267	0.020	0.026	0.019
Roasted moroma bean cake	- 0.688	- 0.286	2.089	- 0.356	- 0.608	- 0.707	- 0.781
Whole moroma bean cake	- 0.822	- 0.179	0.668	0.636	- 0.418	- 0.506	- 0.578

Whole sunflower seedcake	- 0.371	- 0.251	1.122	- 0.471	- 0.419	- 0.478	- 0.543
Crude Protein							
Morama bean cake decorticated	- 0.665	1.660	- 0.973	- 0.516	1.164	1.619	1.721
Roasted morama bean cake	- 0.399	- 0.334	0.871	- 0.114	- 0.119	- 0.269	- 0.166
Whole morama bean cake	0.365	1.144	- 0.253	- 3.600	0.828	0.971	0.873
Whole sunflower seedcake	- 0.592	2.461	- 0.890	- 0.661	1.614	2.272	2.839

$a\%$: Soluble fraction percentage, $b\%$: Slowly degradable fraction percentage, $u\%$: Non-degradable residual amounts percentage, $k_d\%/h$: Fractional disappearance rate per hour, ED2/h, ED5/h, ED8/h: Effective degradability at outflow rates of 2%, 5%, and 8% per hour, respectively, *indicates significant correlation at $p<0.05$

3.5.6 Goodness-of-fit and pairwise comparisons of regression equations between MBCD, RMBC, WMBC and WSSC incubated using *in vitro* and *in situ* techniques

Data comparisons of the goodness-of-fit of the dry matter (DM) and crude protein (CP) regression equations between feeds incubated using *in vitro* and *in situ* techniques are presented in Table 3.14. There were no significant relationships between the DM kinetic degradation fractions (a , b , u , and k_d) of feed components when incubated using *in vitro* and *in situ* techniques. *In vitro*, Daisy^{II} accounted for 50% of the variation in the rapidly soluble fraction (a) when MBCD was incubated in rumen fluid, while the similar fraction from incubation with the *in situ* method accounted for 97%. As for the slowly degradable fraction (b), the fraction considered by the *in vitro* technique was significantly higher ($P<0.05$) by 29% than that obtained by the *in situ* method. The fractional degradation rate (k_d) in the *in vitro* incubation was zero, but it had an average effective DM degradability of 77%, compared to the *in situ* incubation, whose degradation rate was 28%, resulting in an effective DM degradability of 17%.

The a , b , u , and k_d fractions of RMBC determined in the *in vitro* study were overestimated by 32%, 49%, 58%, and 57%, respectively, compared to the *in situ* method. However, the *in vitro* method did not result in meaningful effective DM degradability as the *in situ* method did. No DM degradation fractions were detected during *in vitro* incubation of WMBC, except for the

rapidly soluble fraction, which was 13% higher than *in situ* incubation. The accumulated non-degradable fraction affected the *in situ* degradation rate, resulting in a lower effective DM degradability of 37%.

In vitro incubation of WSSC showed a similar trend to WMBC. The kinetic DM degradation fractions did not show any regression equations. However, *in situ* incubation of WSSC showed DM kinetic degradation fractions, with a , b , u , and k_d accounting for 57%, 36%, 61%, and 96%, respectively. The effective *in situ* DM degradability of WSSC was 59%, which is attributed to the effect of the non-degradable fraction.

The comparison of *in vitro* and *in situ* incubations of the feed components to determine their goodness of fit about their crude protein degradation kinetics is shown in Table 3.15. The kinetic CP degradation fractions of feeds incubated *in vitro* were generally lower ($P < 0.05$) than those of feeds incubated *in situ*, including their effective CP degradation at all levels (Table 3.15).

Table 3.15: Evaluation of Goodness of Fit and Pairwise Comparisons of Regression Equations Between Feeds Incubated Using *In Vitro* and *In Situ* Techniques

		Dry matter			Crude protein		
		<i>In vitro</i>	<i>In situ</i>		<i>In vitro</i>	<i>In situ</i>	
		R ²	R ²	Sig.	R ²	R ²	Sig.
MBCD	<i>a</i>	50.2	97.0	**	49.1	70.4	**
	<i>b</i>	75.4	46.1	**	32.0	41.2	ns
	<i>u</i>	81.0	43.2	**	33.0	56.1	**
	<i>k_d</i>	0.00	28.4	ns	59.0	82.3	**
	<i>ED</i>	77.1	17.2	**	62.2	82.2	ns
RMBC	<i>a</i>	84.1	52.4	**	0.00	62.1	**
	<i>b</i>	93.3	44.0	**	42.1	43.0	ns
	<i>u</i>	92.2	34.2	**	0.00	75.4	**
	<i>k_d</i>	93.1	36.1	**	53.3	67.2	ns
	<i>ED</i>	0.00	90.2	**	0.00	75.2	**
WMBC	<i>a</i>	54.2	41.2	**	0.00	34.1	ns
	<i>b</i>	0.00	44.1	ns	62.0	76.0	ns
	<i>u</i>	0.00	34.4	ns	65.0	56.0	ns
	<i>k_d</i>	0.00	45.0	ns	0.00	55.3	**
	<i>ED</i>	0.00	37.0	ns	64.1	43.1	**
WSSC	<i>a</i>	0.00	57.0	**	78.7	68.6	ns
	<i>b</i>	0.00	36.0	ns	50.7	92.4	**
	<i>u</i>	0.00	61.6	**	52.3	85.1	**
	<i>k_d</i>	0.85	96.5	ns	50.2	71.5	**
	<i>ED</i>	0.00	59.4	**	615	85.0	**

MBCD – *Morona* bean cake decorticated; RMBC – roasted *Morona* bean cake; WMBC – whole *Morona* bean cake; WSSC – whole sunflower seedcake; *a* – rapid soluble fraction; *b* – slowly degradable fraction; *u* – non-degradable fraction; *k_d* – fractional degradation rate; *ED* – effective degradation; **-significant at $P < 0.05$; ns - nonsignificant

3.6 Discussion

3.6.1 Nutrient concentration

Sunflower seed cake is a common protein supplement for ruminants in southern Africa (Ahmad *et al.*, 2004; Al-Dobaib and Kamel, 2012). However, the inherent problem of high protein degradability in the rumen is well-known (Ngongoni *et al.*, 2007; Haro *et al.*, 2019). In a country like Botswana, where sunflowers are not grown in sufficient quantities, this problem of easily degradable proteins is exacerbated, making it imperative to find an alternative protein source for ruminants.

Given the recognized nutritional quality of *Morama* bean cake in human diets (Mmonatau, 2005; Maruatona *et al.*, 2010; Mpotokwane *et al.*, 2013), it was expected that its nutrients would also be relevant to ruminant diets. Therefore, the current study analyzed the chemical and mineral content, fatty acid profile, and DM and CP degradation properties of three different forms of *Morama* (*T. esculentum*) bean cake preparations (MBCD, RMBC, and WMBC) and compared them with sunflower seed cake (WSSC).

The proposed solubilities/insolubilities of the feed fractions (a , b , u , k_d , including ED) of the feed components have been derived from scientifically based calculations and indicate which specific ruminants (cattle) they are suitable for. The solubility or insolubility rate of these feed fractions depends on the chemical composition of the different types of feed.

Among the feeds, roasted *Morama* bean cake (RMBC) exhibited the highest crude protein content (39.2%), followed by decorticated *Morama* bean cake (MBCD; 32.9%) and whole *Morama* bean cake (WMBC; 21.9%). In comparison, the crude protein content of the control oilseed cake supplement (WSSC) was at a similar level to that of WMBC. The CP of the roasted

Morama bean cake is higher than that reported by Múseler and Schonfeldt (2006). The differences between their results and the present results are likely due to differences in processing techniques. The previous authors removed the shells or pods but did not extract oil, while in the current study, the beans were peeled, roasted, and oil extracted.

A study by Jideani *et al.* (2009) found that roasting *Morama* beans increased protein content, confirming the present results. The crude protein content of oil cakes exceeded the acceptable range (7–14% CP-DM basis) for maintenance, production, or reproduction in ruminants (NRC, 1981; Van Soest, 1994; McDonald *et al.*, 2002;). Ruminants do not necessitate highly degradable crude protein (CP) as they are unable to efficiently utilize it due to the activity of rumen microbes (Mlambo *et al.*, 2011).

Morama bean cakes generally had lower NDF, ADF, ADL, DM, OM, and EE values than sunflower seed cakes. However, these chemical properties were within the ranges reported by other researchers (Bower *et al.*, 1988; Museler and Schonfeldt, 2006; Holse *et al.*, 2010) for *Morama*, but were lower in sunflower seed cakes (Ahmad *et al.*, 2004; Mlay *et al.*, 2005; Palmieri *et al.*, 2012). The differences between studies could be related to industrial processing methods, plant varieties, dilution with ether extract, and protein accumulation during growth (Batajoo and Shaver, 1998; Makkar and Becker, 1998; Bamikole *et al.*, 2004; Mlambo *et al.*, 2011; Belachew *et al.*, 2013; Rezaeenia *et al.*, 2014; Gao *et al.*, 2015).

The NDF content of *Morama* bean cake ranged from 26 to 31%, which would ensure the digestibility of the protein supplements (Jolly and Wallace, 2007). NDF plays a crucial role in providing cheap energy (Detmann *et al.*, 2004) and determines dry matter intake (DMI) (Azevedo *et al.*, 2012). Depending on the NDF concentration, it can affect digestion, passage

rate, or retention time (NRC, 2001). NDF content in the range of 45% and above is considered medium quality, while values below 45% are considered high quality (Singh and Oosting, 1992), the latter description being accurate for *T. esculentum* cakes. Generally, feeds with low rumen NDF concentrations are likely to be more digestible (Bakshi and Wadhwa, 2007). Additionally, NDF is associated with the structural components of plant cell walls, which increase in size as the plant matures, providing bulk or fullness to ruminants (Choi and Allen, 1999).

On the other hand, sunflower seed cake had higher DM and NDF values (95.9% and 53.1%, respectively) than those reported by Antunes *et al.* (2019), but lower than those reported by Alves *et al.* (2016). This suggests that it could negatively affect intake in ruminants (Meissner *et al.*, 1991) due to low CP digestion (Arzani *et al.*, 2006; Kamalak *et al.*, 2006). The lower crude protein (CP) utilization in sunflower seedcake by ruminants, despite sufficient CP content, is likely due to the high neutral detergent fiber (NDF) from the seedcoat, which creates a physical barrier, reduces digestibility, increases retention time, leads to microbial competition, and causes an imbalance in nutrient synchronization.

Dias *et al.* (2008) reported that increased NDF in feed leads to low carcass yields because fibre negatively affects the volume of the gastrointestinal tract by limiting feed intake and increasing the time spent on fermentation. Additionally, sunflower seed cake has a lower carbohydrate content than legumes, which may reduce microbial protein synthesis (Ngongoni *et al.*, 2007).

The higher NDF in sunflower seed cake in the current study compared to previously cited studies was likely due to industrial processing techniques, differences in maturity at harvest, the type of cultivar involved, and climatic conditions.

The ADF and CT contents of feed ranged from 10.5% to 21.8% and 1.6% to 4.1%, respectively. These ADF content values are consistent with the findings of Serrapica *et al.* (2019), who reported that various oilseed cakes are valuable alternative protein sources for ruminant diets. The observed cakes in the current study had low ash content and high NFC content, varying between 5% and 10.4% and 18.3% and 41.3%, respectively. The lower ADF and ADL values in *Tylosema* cake indicated prospective high microbial degradability (Kamalak *et al.*, 2005). Feeds with an ADF content of less than 40% are considered of good quality, while feeds with an ADF content of more than 40% are characterized as poor quality (Kellems and Church, 2002).

The lipid content (ether extract) of the oil press cakes ranged from 7.1 to 8.5 g/kg DM, with oleic acid being the predominant fatty acid, especially in *Morama* oil cake. This energy-rich source is utilized by ruminants for maintenance and production (Belachew *et al.*, 2013). Babayemi (2008) reported that the lipid content in the diet of ruminants should not exceed 5%. However, the intake of lipids in the diet of ruminants appears to depend on the feed source, lipid saturation, and the type of animal fed (Martinez *et al.*, 2013).

In the case of *Morama* seed cake, the feed source would not be used as sole feed but as a supplement or in compound feed formulations, thereby balancing fat content with that from other ingredients. Chillard *et al.* (1994) compared the intake of animal dietary fats (688 g/kg DM) with the intake of legume fats (170 g/kg DM) in dairy cows and found that the former influenced low DM intake, but there was no effect with the latter. In a separate study, Pantoja *et al.* (1994) reported that the intake of less than 5% unsaturated dietary fat reduced organic matter (OM) intake and NDF digestibility in dairy cows by 2.8% and 7.6%, respectively.

The deleterious effects of dietary lipids in ruminant diets have been attributed to increased rumination time and slowing of rumen emptying due to disturbances in long-chain fatty acid metabolism (Chilliard, 1993). In general, caution should be exercised when using these oilseed cakes in the diet of ruminants due to their high fat content, which may affect the shelf life and safety of the feed because of secondary products from lipid oxidation (Farias *et al.*, 2019) and the formation of mould (Streit *et al.*, 2012).

The metabolizable energy (ME) of roasted *Morama* bean cake (RMBC) and raw bean cakes (WMBC and MBCD) was 10.7 and 6.91 MJ/kg, respectively, which is lower than the values reported by Museler and Schonfeldt (2006). The authors reported that roasted and raw *Morama* beans had an energy content of 22.8 and 28.0 MJ/kg, respectively. The differences between the current study and theirs are likely due to the amount of oil pressed or extracted and the methods used. Oil extraction increases CP concentration and decreases energy (fat) content (Mlambo *et al.*, 2011). This hypothesis is supported by the results of DePeters *et al.* (1987), who reported that the inclusion of fat in the diet of cows linearly reduced the amount of protein and casein, or vice versa.

Additionally, the process of oil extraction can significantly impact the metabolizable energy of the resulting cake. The removal of oil reduces the overall energy density of the feed, as fats are a concentrated source of energy. This reduction in energy content can affect the feed's suitability for ruminant diets, which require a balanced energy-to-protein ratio for optimal growth and production. Therefore, the lower ME values observed in the current study may be attributed to the higher oil extraction rates, leading to a decrease in the energy content of the *Morama* bean cakes.

The mineral contents of feed ingredients generally met the requirements of growing or productive ruminants (Prabowo *et al.*, 1991), except for potassium content, which was higher (NRC, 2001). This elevated potassium content does not pose a risk to ruminant health and can alleviate heat stress, particularly in tropical regions (Mirzaei, 2012). Similar potassium concentrations in oilseed cakes have been reported elsewhere (Prabowo *et al.*, 1991). The ionic nature of potassium in feed enhances microbial attachment to feed substrates (Van Soest, 1994), translating to better feed utilization.

Ruminants require a balanced intake of both macro and micro minerals for optimal health and productivity. Macro minerals, including calcium, phosphorus, magnesium, potassium, sodium, chlorine, and sulfur, are needed in larger amounts and play crucial roles in bodily functions such as bone formation, muscle contraction, and enzyme activation. Micro minerals, or trace elements like cobalt, copper, iodine, iron, manganese, selenium, and zinc, are required in smaller quantities but are essential for various metabolic processes, immune function, and reproduction.

The mineral content of feed ingredients should be carefully managed to avoid deficiencies or toxicities, which can negatively impact animal health and productivity. For example, an imbalance in calcium and phosphorus can lead to metabolic disorders, while excess selenium can cause toxicity. Regular feed analysis and appropriate supplementation can help ensure that ruminants receive the necessary minerals for their specific needs.

Diets fortified with *Morama* bean cake generally have sufficient micromineral content (0.01%), but the manganese content is higher than the recommended value (0.04%). Previous studies (Prabowo *et al.*, 1991; Khan *et al.*, 2007) reported lower manganese levels in oil cakes and

animal feed. Elevated manganese levels are often associated with low cobalt (Co) levels in soil due to the antagonistic effect between the two minerals (Khan *et al.*, 2007). Although the cobalt content was not measured in this study, it is hypothesized that soil cobalt levels could influence manganese uptake.

Ideally, oil cakes are used as part of a balanced animal ration rather than being fed alone. This approach dilutes the high mineral concentrations in the oil cake with other feed ingredients, ensuring an acceptable level in the overall diet.

3.6.2 *In vitro* Fermentation

The oilseed cakes in the current study are characterized by a high concentration of hemicellulose, cellulose, and a certain proportion of residual oil, as well as a higher NDF, especially in the sunflower cake. This results in different degrees of degradability, associated with slow to fast soluble fractions and high degradation rates. To the best of the author's knowledge, there is limited, if any, data on the degradation kinetics of *Morama* oil cakes, and no literature on the degradation kinetics of *Morama* oil cakes has been published. Among all oil cakes, the highest soluble fraction was observed in *Morama* bean cake decorticated (MBCD), with WMBC having the lowest. This suggests that *Morama* oil cake can be integrated into the diet of ruminants and can partially replace some of the conventional oil cakes.

3.6.3 *In vitro* Dry matter disappearance

After 24 hours, *in vitro* asymptotic analysis showed an obvious disappearance of DM by 69% for MBCD, followed by RMBC, WMBC, and WSSC with 51.3%, 36.5%, and 32.9%, respectively, consistent with the digestibility of oil cake (Xu *et al.*, 2010). The differences between MBCD and other feedstuffs in terms of degradability were due to the lack of a seed

coat that could have prevented the action of microbial enzymes. This was also consistent with samples with lower rumen NDF; feeds with lower NDF are more easily degradable (Van Gelder *et al.*, 2005).

In a study by Defeo *et al.* (2019) on the differences between decorticated barley and conventional intact barley in rumen degradation, they reported that decorticated barley was sufficiently degradable due to the reduced concentration of NDF and ADF (cellulose and lignin), which explains the loss of external fibres with MBCD. The effective DM degradation of MBCD was higher than other oil cakes (RMBC, WMBC, and WSSC), which is explained by its higher degradation coefficient. The effective DM degradability of MBCD and RMBC was inconsistent with the observations of Bruno-Soares *et al.* (2000), who reported that NDF, ADF, ADL, and *b* fraction in feed have inherent positive correlations. However, the differences in diets were attributed to the soluble and insoluble but rumen degradable fractions, both of which were higher in MBCD than in other oil cakes.

The effective DM of WSSC was close to the value recorded in MBCD but higher than both WMBC and RMBC. However, this result was obtained with different kinetic parameters. Although the 'b' fraction was similar in WSSC and RMBC, the 'a' fraction was higher in WSSC and lower in RMBC and WMBC, while the DM disappearance rate was higher in RMBC and lower in both WSSC and WMBC. Therefore, the non-degradable fraction (*u*) of feed ingredients was presented in descending order as WMBC > RMBC > WSSC > MBCD. This order is explained by the increased amount of fiber from the seed coat (cellulose and lignin) of the WMBC, which is also associated with condensed tannin (Defeo *et al.*, 2019), heat-promoted peptide chain cross-link rearrangements, and chemical bonds with carbohydrates (Heim and Krebs, 2019) in the RMBC. High degradability due to seed coat loss

(Xu *et al.*, 2010) and high N-solubility (Ngongoni *et al.*, 2007) impacted MBCD and WSSC. These problems associated with WMBC and RMBC may affect the potential degradability of feed by rumen microbes (Repetto *et al.*, 2003).

Thus, dry matter (DM), particularly organic matter, determines the rate at which the rapidly soluble portion (*a*) and a potentially degradable portion (*a+b*) of the feed components can deliver nutrients to the rumen microbes and the host animal. However, this would only be beneficial to ruminants if the NDF structural cell wall content is as low (Singh and Oosting, 1992) as that observed in *Morama* oilcakes. Although the rate of dry matter (DM) disappearance in this study was only measured for 24 hours, as suggested by Ørskov (1992), the results were quite impressive. Particularly, MBCD and RMBC demonstrated increased DM degradability over time, surpassing the obligatory minimum of 50% lag (Perez *et al.*, 2001).

The *in vitro* dry matter disappearance (IVDMD) technique is a valuable method for estimating the digestibility of feedstuffs. It involves incubating the feed samples in a simulated rumen environment to measure the extent of degradation over a specified period. This method provides insights into the potential nutritional value and effectiveness of feed ingredients in ruminant diets. IVDMD can be influenced by various factors, including the chemical composition of the feed, the presence of anti-nutritional factors, and the physical structure of the feed particles. Feeds with high fibre content, particularly NDF and ADF, tend to have lower IVDMD values due to the resistance of fibrous components to microbial degradation. Conversely, feeds with lower fibre content and higher solubility, such as MBCD, exhibit higher IVDMD values, indicating better digestibility.

However, the presence of seed coats or other structural barriers can impede microbial access to the feed particles, reducing the overall degradability. Removing these barriers, as observed with decorticated feeds like MBCD, enhances the exposure of feed particles to microbial enzymes, resulting in higher degradation rates. Additionally, the preparation methods, such as roasting or oil extraction, can affect the chemical composition and physical structure of the feed, influencing its degradability.

In the context of ruminant nutrition, understanding the IVDMD of feed ingredients is crucial for formulating balanced diets that optimize nutrient utilization and animal performance. High IVDMD values indicate that the feed can be effectively broken down in the rumen, providing a steady supply of nutrients to the animal (Pratama *et al.*, 2022). This is particularly important for high-producing ruminants, such as dairy cows and beef cattle, which have higher nutritional requirements. Overall, the results of the current study highlight the importance of considering both chemical composition and physical structure when evaluating the nutritional value of feed ingredients. The high IVDMD values observed for MBCD and RMBC suggest that these feedstuffs have the potential to enhance nutrient utilization and support optimal growth and production in ruminants.

3.6.4 In vitro crude protein disappearance

After 24 hours, *in vitro* graphic analysis showed an obvious disappearance of CP by 97.2% in MBCD, followed by WSSC, WMBC, and RMBC at 90.8%, 49%, and 45%, respectively. This is consistent with the CP digestibility of groundnut (*Arachis hypogaea*) oil cake (Ørskov, 1992; Defeo *et al.*, 2019). Crude protein disappearance (CPD) values differed between feed ingredients due to oil concentration, processing, and inherent protein solubility properties that facilitated attachment to rumen microbes (Promkot *et al.*, 2007).

A preliminary laboratory study by Kgwadi (2021, *unpublished*) found that the protein solubility of *Morama* bean cake decorticated (56.4%) was similar to that of sunflower seed cake (49.3%) and higher than that of *Marula* seed cake (31.9%) and *Mophane* flour (27.3%). In the current study, the highest proportion of CPb detected in MBCD could be explained by its chemical composition: low ADF and NDF, low CT, and high CP content, resulting in the highest effective crude protein degradability compared to other protein sources. However, reduced effective CP degradability might be related to negative sample characteristics (Repetto *et al.*, 2003).

Surprisingly, RMBC showed the highest NDF degradation rate (0.320/h). Heat treatment typically increases the level of neutral detergent insoluble protein (NDICP) in the feed (Mustafa *et al.*, 2003), although this value can increase or decrease depending on nitrogen content (McKinnon *et al.*, 1990). RMBC had the highest nitrogen content, which explains why its NDF degradability increased (Van Soest, 1994). Demjanec *et al.* (1995) reported a similar increase in NDF degradability after low-heat soybean and rapeseed mill treatments. However, Ndlovu and Nherera (1997) suggest that there may be a negative correlation between cell wall contents and CP degradation rates. This aligns with the current study's findings when comparing the *in vitro* technique with the *in situ* method in the degradability of whole *Morama* bean cake (WMBC). The results for MBCD and WSSC in the present study indicated that their crude protein could be highly hydrolyzable in the rumen. Such degradation could lead to excess ammonia and asynchrony between fermentable energy and protein, which could lead to underutilization of the feed ingredient (Firkins and Reynolds, 2005).

As mentioned above, the lower effective CP degradation abilities of WMBC and RMBC could be due to the formation of tannin-protein complexes (TPC) from the seed coat and heat treatment, respectively (Wanapat *et al.*, 2000). WMBC showed a condensed tannin content of

4.1% DM, and the available literature (Mueller-Harvey, 2006; Theodoridou *et al.*, 2010; Jayanegara *et al.*, 2015) indicates that a CT content of 5 g/kg or less advantageously binds protein that could subsequently dissociate in the acidic environment of the abomasum and release the protein, which is then easily digested in the small intestine (McNabb *et al.*, 1996). Jones and Mangan (1977) reported that pH below 3 and above 8 results in the dissociation of the tannin-protein complex.

In contrast, MBCD and WSSC showed higher effective CP degradability (86.5% and 76.3%, respectively) than the National Research Council (NRC, 2001) recommended value of 29.2% for concentrates, a condition that may require CP protection in the rumen environment (Putri *et al.*, 2021). The high loss of N-solubility in sunflower seed cake was consistent with the report of Ngongoni *et al.* (2007), in which the authors stated that it was 85% soluble. The rapidly soluble fractions contained significant amounts of total protein (non-protein nitrogen (NPN) and true protein). These fractions are intended for use by rumen microbes (NRC, 2001). Regardless of heat treatment, RMBC had faster CP degradation rates at all incubation times due to the high nitrogen content (Gurbuz, 2007) and would thus benefit the rumen microbes (Ørskov, 1992).

The *in vitro* crude protein disappearance (IVCPD) of feed ingredients is influenced by several factors, including their chemical composition, the presence of anti-nutritional factors, and the physical structure of feed particles. Feeds high in fiber content, such as NDF and ADF, typically exhibit lower IVCPD values due to the resistance of fibrous components to microbial degradation. Additionally, processing methods like roasting or oil extraction can modify the chemical composition and physical structure of feed, thereby affecting its degradability.

In ruminant nutrition, understanding the *in vitro* crude protein degradability (IVCPD) of feed ingredients is essential for developing balanced diets that optimize protein utilization and enhance animal performance. High IVCPD values indicate that protein can be efficiently degraded in the rumen, ensuring a continuous supply of amino acids to the animal. This is especially crucial for high-producing ruminants, such as dairy cows and beef cattle, which have elevated protein requirements for milk production, growth, and reproduction.

Overall, the findings of this study emphasize the importance of considering both the chemical composition and physical structure of protein sources when evaluating their nutritional value. The elevated IVCPD values observed for MBCD and WSSC suggest that these feedstuffs can significantly improve protein utilization and support optimal growth and production in ruminants. However, the high degradability of their proteins may compromise amino acid sustenance.

3.6.5 *In situ* dry matter disappearances

In situ incubation of diets showed similar DM disappearance patterns as in the previous *in vitro* study. The overall variation in oilseed press cake, especially after zero hours, was faster due to microbial degradation (Singh *et al.*, 1989; Khan *et al.*, 2009). The disappearance characteristics were consistent with the results of other researchers (Hoffman *et al.*, 1993; Kamalak *et al.*, 2005; Kamalak, 2006), who reported that DM disappearance was correlated with CP content. Another important factor that may have contributed to the disappearance of high DM levels was the presence of non-fiber carbohydrates (NFC), particularly in RMBC, MBCD, and WSSC, which benefited the rumen microbes. The presence of carbohydrates complements the utilization of Ammonia-N by rumen microbes (Tylutki *et al.*, 2008). Belachew *et al.* (2013) reported that higher concentrations of NDF and CT have a negative impact on the

disappearance of DM because more rumen microbes may be inactivated or inhibited by these chemicals. In contrast, WMBC had low NDF content but high CT content, resulting in very low disappearance rates. This suggests that CT was more effective in inactivating microbes.

3.6.6 *In situ* crude protein disappearances

The disappearance of CP at zero hours (a-fraction) was highest in WSSC and RMBC, at 38.1% and 35%, respectively. These findings demonstrate the readily available nitrogen supply to rumen microbes provided by WSSC and RMBC. The same protein diets achieved greater than 80% CP degradability within 24 hours of incubation. This finding aligns with Manju *et al.* (2022), who found that the slow-release non-protein nitrogen sources achieved greater than 80% crude protein (CP) degradability within 24 hours when fed to Deccani rams. The CP disappearance properties of the oilcakes are attributable to the solubility of their proteins.

Whetton *et al.* (1997), cited in Belachew *et al.* (2013), reported that different protein sources with different structural carbohydrate contents may disappear differently. The authors further explained that the combination and concentration of carbohydrates determine the extent to which CP becomes soluble and bioavailable.

The low CP disappearance values at zero hours could be due to the action of prolamin and condensed tannin (Van Soest, 1994). Prolamins are protein fractions characterized by high levels of glutamine and proline, both of which are hydrophilic (Van Soest, 1994). As previously mentioned, MBCD likely contains prolamin and would therefore delay microbial colonization in the rumen (Momany *et al.*, 2006).

In WMBC, the condensed tannin content (4.1%) was slightly lower than the 5.5% reported by Aganga and Tshwenyane (2003). Coupled with an increased oil content (Babayemi, 2008), this

could be the reason why it was not rumen-degradable. The differences in chemical composition between the study by Kgwadi (2021) and the present study highlight the variations between laboratories in sample preparation and processing methods, as well as in the characteristics of the apparatus or bags used for incubation.

3.6.7 *In Situ* Drymatter Degradability Characteristics

The effective dry matter degradability (EDMD) was derived from the estimated degradation properties. This was based on assuming rumen flow rates of 0.02/h (maintenance fed), 0.05/h (medium production fed), and 0.08/h (high production fed ruminants). The commonly observed values for the highly soluble fraction (a) and the less rumen-slowly degradable but potentially degradable fraction (b) of oilseed cake could be due to losses attributed to the properties of the nylon bag rather than microbial action. However, the non-degradable residue (u) was higher in MBCD. This could be due to the inherent prolamin content (Bower *et al.*, 1988) of *Morama nut*. According to Bower *et al.* (1988), cited in Mmonatau (2005), prolamin is an alcohol-soluble protein that is rare in legume seeds but abundant in *Morama* nuts (Museler and Schnfeldt, 2006). The protein interferes with microbial colonization and thus prevents the breakdown of dry matter (Momany *et al.*, 2006).

WMBC showed average DM degradability due to its condensed tannin content. Its condensed tannin content was 4.1 g/kg DM. Condensed tannins (CT) can bind protein (Kumar and Singh, 1984) and inhibit microbial degradation (Makkar *et al.*, 1989; Mueller-Harvey, 2006), especially when the CT content of the feed is above 4 g/kg DM (Aganga and Tshwenyane, 2003). However, it is observed that lower CT values (<4 g/kg DM), as seen in RMBC (2.3 g/kg), MBCD (3.5 g/kg), and WSSC (1.5 g/kg) (DM basis), can improve amino acid flow in the small intestine (McNabb *et al.*, 1996).

3.6.8 *In Situ* Crude Protein Degradability Characteristics

The study observed significant differences in crude protein (CP) degradation parameters between sunflower cake and *Morama* cake, as well as within different legume cakes. Roasted *Morama* bean cake (RMBC) demonstrated 18.3% higher mean values for the fast CP-soluble fractions (*a*) compared to *Morama* bean cake decorticated (MBCD; 16.7%), whole *Morama* bean cake (WMBC; 6.3%), and whole sunflower seed cake (WSSC; 13%).

The results indicate substantial variations in the degradation parameters and effective degradability (ED) of CP among the different feed treatments. RMBC generally exhibited higher values for the *a* and *b* fractions, indicating a greater potential for CP degradation in the rumen. The k_d fraction values varied, with MBCD and WSSC having the highest rates. The ED values at different outflow rates showed that RMBC consistently had higher degradability, particularly at lower outflow rates (0.02/h), suggesting its superior digestibility. Conversely, WMBC exhibited the lowest values across all parameters, indicating lower digestibility.

Contrary to the assumption that the non-fibrous carbohydrates (NFC) fermented by the rumen microbes influence the fast soluble fractions of the feed parameters (Antunes *et al.*, 2019), this was not the case with RMBC feed ingredients, although RMBC had lower NFC (18.3%) than MBCD (33.6%). Furthermore, the degradability of the slowly degradable fraction (*b*) depends on the NDF content of the feed, mainly its hemicellulose and cellulose (Antunes *et al.*, 2019). Yang and Russell (1995) reported that the degradability of dietary protein depends on the type of bonds involved in the architecture of the protein molecules after roasting. The results of the current study on CP fraction degradation are consistent with those observed by some researchers (Narayana and Narasinga-Rao, 1985; Yusuf *et al.*, 2008). This group of researchers reported that beans tended to have increased water-holding capacity when roasted/heated.

WMBC had the lowest potential degradability due to its inherent condensed tannin content, which had a negative impact on the microbes. Not surprisingly, the CPb degradability of WSSC was lower than that of RMBC, which was attributed to the higher NDF content (53.1% vs. 31.4%). The higher the NDF content of the feed, the lower its digestibility and the reduced voluntary intake of feed in ruminants.

RMBC had the highest effective crude protein degradability (EDCP) at all rumen outflow rates (2%, 5%, and 8%), while WMBC had the lowest. The lower effective degradability of WMBC may be related to its lipid/oil content, which may have resulted in clogging bag pores or inactivating rumen microbes, thereby slowing the degradation process (Goes *et al.*, 2011). WMBC was never peeled, and the pulverized shells may have mixed with the oil, making it difficult for the particles to pass through the nylon bag net (Moreira *et al.*, 2003) or for the microbes to migrate into the bags. Fortaleza *et al.* (2009), cited in Antunes *et al.* (2019), reported a similar observation when working with cotton seeds with an ether extract content of 15.5%.

The interaction of ester bonds between lignin and hemicellulose impaired the microbial degradation of rumen cell walls and affected the utilization potential of nutrients due to the asynchrony of protein and energy in the rumen (Antunes *et al.*, 2019). The lignin and condensed tannin contents of WMBC were the highest among the oilseeds (1.3% and 4.1%, respectively). The size of the remaining non-degradable protein pool (u) of the WMBC could be the reason why the oilseed cake had greater potential CP indigestibility due to the inactivation of rumen microbial activity (Antunes *et al.*, 2019).

3.6.9 Relationships Between Chemical Components and Rumen Dry Matter (DM) Degradation Parameters

The disappearance of dry matter (DM) *in situ* and other estimated degradation parameters showed positive correlations with organic matter (OM), crude protein (CP), acid detergent fiber (ADF), and condensed tannin (CT) in *Morama* bean cake decorticated (MBCD); OM and CP in roasted *Morama* bean cake (RMBC); ADF in whole *Morama* bean cake (WMBC); and CP, ADF, and acid detergent lignin (ADL) in whole sunflower seed cake (WSSC). According to the present study, the degradability of neutral detergent fiber (NDF) and ADF were positively correlated with CP content but negatively correlated with NDF, ADF, and lignin content. This result aligns with Hoffman *et al.* (1993), who reported that *in situ* NDF degradability is positively correlated with CP content. OM and CP correlated with the dry matter soluble (a) and insoluble (b) fractions of the oilseed cakes. These relationships applied to MBCD, RMBC, and WSSC. The weaker relationship of WMBC was due to the effect of protein-condensed tannin complexes (Beuvinik and Kogutk, 1993; Gonzalez *et al.*, 2002; Gupta *et al.*, 2011), resulting in low effective DM degradability of the oilseed cake. The current study's observation concurs with the findings of other researchers (Tolera *et al.*, 1997; Abdulrazak *et al.*, 2002).

Furthermore, lignin or the ratio of lignin to NDF implies greater lignification of the cell wall and would logically reduce the degradability of the fibres. As a result, NRC (2001) predicts the degradability of fibres based on the ratio of lignin to NDF. Nousianinen *et al.* (2004) argued that there is no strong relationship between fiber degradation characteristics and the ratio of lignin to NDF. However, in their study, they observed that neutral detergent fiber degradability (NDFD) and acid detergent fiber degradability (ADFD) had a medium correlation ($R^2 = 0.54 - 0.67$) with lignin content.

The *in situ* crude protein degradability (CPD) technique is vital for evaluating the degradability of protein in feedstuffs under conditions that closely mimic the rumen environment. This method involves placing feed samples in small, porous nylon bags and incubating them in the rumen of live ruminants. It provides critical insights into the dynamics of protein degradation and the interaction between feed components and rumen microbes.

The significance of *in situ* CPD in ruminant nutrition is attributed to the three factors:

- **Nutrient Supply:** Accurate estimation of CPD helps formulate diets that provide a steady supply of amino acids to support growth, milk production, and reproduction in ruminants.
- **Feed Efficiency:** Understanding CPD allows for optimizing feed utilization, reducing waste, and improving the overall efficiency of protein use in ruminant diets.
- **Animal Health:** Properly balanced diets with appropriate CPD can help prevent metabolic disorders and support the overall health and productivity of ruminants.

In summary, *in situ* CPD is a valuable tool for evaluating the degradability of protein in feedstuffs. The current study's findings on the CPD of various *Morama* bean cakes and sunflower seed cake highlight the importance of considering chemical composition, processing methods, and physical structure when formulating ruminant diets. The higher CPD values observed for RMBC and MBCD suggest that these feedstuffs have the potential to enhance protein utilization and support optimal growth and production in ruminants.

3.6.10 Optimal Combinations of Chemical Components for Predicting Rumen DM Degradation Parameters Using Backward Stepwise Multiple Regression

The dry matter (DM) degradation kinetics of the oilseed cakes were based on several obvious correlations with chemical constituents. The regression was performed for the prediction equation values of the dry matter observed in the feeds, taking into account chemical properties and their bio kinetic degradation, including effective degradability. However, it is unlikely that the values measured *in situ* or *in vitro* are identical to the *in vivo* values. Therefore, mathematical models are used to link data obtained *in vitro* or *in situ* with processes occurring *in vivo* (Lopez, 2005).

The non-degradable (u) fraction of dry matter (DM) and the effective DM degradability of sunflower seed cake (WSSC) at a rumen outflow rate of 0.02 per hour were predicted by condensed tannin (CT) and crude protein (CP) with determination coefficients (R^2) of 0.611 and 0.534, respectively. This observation indicates that WSSC is easily fermented and can be considered an additional energy source for rumen microbes due to its high content of non-fibrous carbohydrates (189 g/kg in DM). Furthermore, at various R^2 values of 0.535, 0.594, and 0.644, CP predicted at effective DM degradability levels of 0.02, 0.05, and 0.08 per hour (EDDM2, EDDM5, and EDDM8), respectively, were highly correlated with potential *in vivo* CP values for WSSC (Vasudha and Sarla, 2021).

The significant disappearance of CP in WSSC during incubations using either technique (*in vitro* or *in situ*) could be used to estimate its CP degradability and susceptibility to rumen microbes (Kamalak *et al.*, 2004). However, the *in situ* technique should be used with caution when estimating the nutritive value of feed containing a certain level of phenolics, such as *Tylosema* or *Helianthus* cakes, as the potential negative effects on rumen microbes may not be detected. In contrast, *in vitro* studies consistently demonstrate the inhibitory effects of phenolics in feeds (Kamalak *et al.*, 2004). Additionally, the *in vitro* method is a closed system

with a limited supply of rumen fluid, making it easier for any anti-nutritive compound to affect the rumen microbes. The *in situ* technique, on the other hand, is an open system with a much wider environment and ample rumen fluid supply to the bags, resulting in less detection of anti-nutritive effects (Kamalak *et al.*, 2004).

3.6.11 Optimal Combinations of Chemical Components for Predicting Rumen CP Degradation Parameters Using Backward, Stepwise, Multiple Regression.

The rumen degradation proportions of crude protein (CP) in oilseed press cakes at different rumen incubation times were influenced by the chemical compositions of the feed samples. The non-degradable crude protein (uCP) had a negative association with the CP content of the oilseed cakes. Ideally, the high crude protein content of oilseed cake would provide more nitrogen to rumen microbial cells. However, the amount of remaining undegraded crude protein fractions would depend on the digestibility of the crude protein in the lower gastrointestinal tract (Ali *et al.*, 2014). Although the current study did not determine the protein classification of *Tylosema*, Müsseler and Schönfeldt (2006) reported that it contains 15% prolamin relative to its nut size. This could have implications for the degradability or digestibility of its bean cake in the rumen. Proteins such as prolamins and glutelins are insoluble and slowly degraded in the rumen, whereas globulins are soluble and easily degraded (Romagnolo *et al.*, 1994). Based on the coefficient of determination (R^2) and the probability value (PV) in the present study, condensed tannin (CT) was identified as the best single predictor of the effective degradation of crude protein at an 8% per hour rumen outflow rate (EDCP8) observed in MBCD. This likely explains why the feed ingredient did not exhibit critical requirements for rumen degradability traits.

3.7 Conclusion

The ruminal dry matter (DM) and crude protein (CP) degradability of *Morama* bean cakes (WMBC, RMBC, and MBCD) and sunflower seed cake (WSSC) were estimated using *in vitro* and *in situ* methods. *Morama* bean cake exhibited lower DM and CP degradability values available to rumen microbes compared to sunflower seed cake, which could have significant implications for livestock feed efficiency and overall animal health. Special mention goes to roasted *Morama* bean cake (RMBC), which has lower dry matter disappearance. It provides a slower, more sustained release of nutrients, benefiting stable rumen conditions and preventing digestive disturbances. The roasting process impairs its effective crude protein degradability, suggesting lower intestinal digestibility. This characteristic complements the amino acids provided by rumen microbes.

Further studies are required to determine the synergistic effects of the bean cake and the type of forage to be mixed with for ruminants, and these must be carried out *in vivo*. Since the feed ingredients tested in the present study are protein sources, it is important to first formulate rations from locally available basal diets fortified with the correct energy levels and test these rations for their suitability in ruminants.

Based on the degradation kinetics of the feed samples, both methods (*in vitro* and *in situ*) were consistent, except for the effective DM and CP degradation parameters of *Morama* bean cakes in the *in vitro* technique. *In situ* rumen incubation, which involves placing samples directly into the rumen of a live animal, often provides more accurate and reproducible results because it closely mimics the natural environment of the rumen. This method can account for the complex interactions between the rumen microbes and the host animal.

On the other hand, *in vitro* techniques like the Daisy^{II} incubation system use rumen fluid collected from donor animals to simulate the rumen environment in a laboratory setting. While this method is more controlled and less invasive, it may not capture all the nuances of the *in vivo* rumen environment, potentially leading to less reproducible results. Therefore, it is expected that the heat-treated protein fractions *in situ* will offer more amino acids to the small intestine *in vivo* if they are sufficiently available. Moreover, the high *in vitro* dry matter disappearance (IVDMD) values observed for *Morama* bean cake decorticated (MBCD) and roasted *Morama* bean cake (RMBC) suggest that these feedstuffs have the potential to enhance nutrient utilization and support optimal growth and production in ruminants.

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Chapter 4

CHEMICAL COMPOSITION, *IN VITRO* RUMINAL DEGRADABILITY AND ABOMASAL DIGESTION OF RICE STRAW SUPPLEMENTED WITH ROASTED *MORAMA* BEAN CAKE OR WHOLE SUNFLOWER SEED CAKE

ABSTRACT

The use of roasted *Morama* bean cake (RMBC) could provide higher nutritional value as an alternative protein supplement for ruminants. This study aimed to evaluate the protein supplementation potential of roasted *Morama* bean cake (*Tylosema esculentum*) compared to whole sunflower seed cake (*Helianthus annuus*) when added to rice straw. The oilseed cakes were mixed with milled rice straw. The substrate formulation described above was subjected to a t-test or independent comparison to evaluate differences between RMBC and WSSC at identical supplementation levels (0%, 10%, 20%, and 30%). The experimental setup followed a completely randomized design (CRD) matrix, with three replicates per treatment combination. The diets underwent pre-analysis for dry matter (DM), crude protein (CP), ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), cellulose, hemicellulose, and non-fiber carbohydrates prior to incubation with rumen inoculant in the Daisy^{II} device. The incubation of treatments was conducted in two stages: *in vitro* in the rumen (48 hours at 39°C for stage I) and in the post-ruminal step (stage II; pepsin-HCl, lasting 24 hours). The inoculum for *in vitro* incubation was obtained from a Tswana steer equipped with a permanent rumen fistula. The *in vitro* residues of crude protein degradability (*iv*ACPdeg) were determined as CP disappearance minus bypass crude protein (BP-CP).

The mixture of rice straw with various levels of oilseed cakes had a significant ($P<0.05$) influence on dry matter (DM), crude protein (CP), ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), cellulose, hemicellulose,

and non-fiber carbohydrates. The incubated feed treatments differed significantly ($P<0.001$) in pH, ammonia (NH_3), and total volatile fatty acid production. With increased incorporation of oilseed cake, the protein content of rice straw, its nutrient degradability or nutrient digestibility, ammonia concentration, total volatile fatty acid, and microbial protein synthesis were uniformly increased ($P<0.0001$). *In vitro* true digestible dry matter degradability (*ivTDMdeg*), *in vitro* organic matter degradability (*ivOMdeg*), and *in vitro* apparent dry matter degradability (*ivADMdeg*) increased linearly ($P<0.0001$) when 30% RMBC was added to rice straw. In stage II, apparent *in vitro* crude protein digestibility (*ivACPdig*), *in vitro* apparent dry matter digestibility (*ivADMdig*), and bypass crude protein digestibility (BP-CPdig) were 16.2%, 56.5%, and 31.6%, respectively, and were higher ($P<0.05$) for rice straw supplemented with 30% roasted *Morama* bean cake than when supplemented with 30% whole sunflower seed cake.

Based on the current study results, it was concluded that the inclusion of 30% RMBC in the rice straw could optimize the productivity of ruminants by providing better protein flow to the abomasum.

Keywords: bypass crude protein digestibility; *in vitro* apparent crude protein; bypass crude protein; *in vitro* apparent dry matter digestibility; *in vitro* apparent dry matter degradability

4.1 Introduction

Tylosema esculentum (*Morama*) is a less studied nut/bean legume from southern Africa. The average crude protein content of *Morama* bean is estimated to be 37% (Kgwadi, 2021, *unpublished*), and it is rich in unsaturated fatty acids and phytochemicals such as phenols, flavonoids, saponins, and phytosterols (Khan *et al.*, 2019; Saxena *et al.*, 2013). Dietary protein provides a direct source of essential amino acids, whereas non-protein nitrogen (NPN) relies

on microbial conversion (Alcaide *et al.*, 2003). Therefore, rumen microbes require preformed amino acids. Providing true protein in the diet can enhance overall nutrient utilization and feed efficiency in ruminants (Putri *et al.*, 2021). This can result in better growth, reproduction, and milk production. *Morama* bean is currently viewed as a potential source of good quality protein. Nevertheless, there is currently no information on the extent of the quality and quantity of additional protein that *Morama* bean cake, let alone roasted *Morama* bean cake, could provide to ruminant diets.

However, Study 1 showed that roasted *Morama* bean cake has good chemical properties with better dry matter (DM) and crude protein (CP) degradation properties in the rumen than the other two *Morama* forms. Botswana, like most semi-arid countries, experiences extremely adverse conditions such as erratic rainfall followed by long droughts, resulting in the loss of pastureland. While there is plenty of palatable and digestible fodder for ruminants in the rainy season, both the quantity and quality of this feed decrease as the dry season begins. Under such limited feed conditions, ruminants are then forced to use poorly grown hay or unconventional leftover feeds. Plant residues therefore play an important role in animal production systems as they can be harvested and utilized by animals. The only disadvantage of crop residues, particularly grain-based ones, is their low nitrogen content and limited rumen decomposition. Botswana has recently experimented with growing rice under dryland conditions.

To increase the nutritional value of rice straw, it must be processed before feeding to ruminants. It is often subjected to physical, chemical, or even biological interventions. Many researchers (Trisnadewi *et al.*, 2011; Lamid *et al.*, 2013; Mustabi *et al.*, 2013) have tried to improve the quality of rice straw using various methods, including fermentation. Fermentation provides benefits such as enhancing the taste of the feed and improving its nutritional quality and palatability (Sarnklong *et al.*, 2010). However, it was found that these improvements were not

sufficient to meet the minimum nutritional requirements of ruminants (Natsir, 2012). Therefore, rice straw requires high-quality protein supplements to maximize ruminant production, but the rising prices of concentrates have always discouraged small-scale farmers. It is always advisable to use available alternative concentrates.

Sunflower seed cake has long been the most popular protein supplement for ruminants in Botswana, despite being easily degradable in the rumen (Ngongoni *et al.*, 2009; Calsamiglia *et al.*, 1997). However, *Morama* nuts appear to be a potential alternative protein source for ruminants. There is no equivalent ruminant evaluation work comparable to Manyeula *et al.* (2013)'s poultry work on *Morama*. The starting point for evaluating such feed protein sources (roasted *Morama* bean cake) and crop residues (rice straw) for ruminants is therefore to carry out *in vitro* tests. Consequently, an *in vitro* study using rice straw supplemented with roasted *Morama* bean cake was conducted in the present study. Due to its beneficial properties, roasted *Morama* cake was selected based on the results of Study 1.

Thus, this study evaluated the supplemental potential of roasted *Morama* bean cake and its effects on the production of volatile fatty acids, ammonia, and fluctuations in rumen pH. The study compared these oilseeds press cakes: roasted *Morama* bean cake (*Tylosema esculentum* L.) and sunflower seed cake (*Helianthus annuus* L.) in rice straw diets. It was hypothesized that roasted *Morama* bean cake would improve the rumen degradability of rice straw and increase the supply of rumen non-degradable protein. This hypothesis was examined by comparing the chemical composition, *in vitro* ruminal dry matter (DM) degradability, *in vitro* crude protein (CP) degradability, by-pass crude protein, by-pass crude protein digestibility, post-ruminal DM digestibility, volatile fatty acids (VFAs), and ammonia concentration of rice straw supplemented with roasted *Morama* bean cake compared with whole sunflower seed cake at different levels of inclusion.

4.2 MATERIALS AND METHODS

4.2.1 Ethical Approval

The techniques and treatments used in this study were conducted in accordance with the Environmental Ethics in Experiments and Animal Ethics (2019). Approval was requested and granted by the BUAN Animal Ethics Committee (BUAN-AE-2022-05).

4.2.2 Study Site Description

The research was conducted at the Botswana University of Agriculture and Natural Resources (BUAN) Animal Nutrition Laboratory and the Department of Agricultural Research (DAR) at Sebele Content Farm. The study material (rice straw) was obtained from the university farm at longitude 25.9413°E and latitude 24.5912°S. The climatic conditions in Sebele, Botswana, are subtropical-semi-arid, temperate depending on altitude, with a hot rainy season from November to March (precipitation can be up to 485 mm per year) and a long dry season from April to October, with a cool period from May to August. During the cool period, the temperature can drop a few degrees below freezing at night. From September to April, it can be very hot during the day. Sebele is located 9 km from the city of Gaborone, which is situated in the southeastern part of the country at 1000 meters above sea level (Botswana Meteorology, 2021).

4.2.3 Feeds and substrates

The following oilseed press cakes were used in this study: roasted *Morama* bean cake (*Tylosema esculentum* L.), sunflower seed cake (*Helianthus annuus* L.), and the basal diet was rice straw (*Oryza sativa* L.). The sunflower seeds and *Morama* beans were purchased from Agri-Feed (a local dealer) and farmers in Maboane village (Kweneng district), respectively.

Rice straw (RS) was used as the basal diet, while whole sunflower seed cake (WSSC) and roasted *Morama* bean cake (RMBC) provided protein supplements in varying amounts.

The rice straw used was a composite derivative of three introduced drought-tolerant rice varieties, namely AA 073, AA 212, and AA 128 from China. Rodgers (2021; *unpublished*) reported that there was no difference in crude protein and *in vitro* digestibility content between these varieties. They had a mean CP of 9.54%, an *in vitro* digestibility of 69.34%, but variable ADL values (24.09%, 28.07%, and 26.26%), NDF (11.31%, 20.45%, and 15.95%), and ADF (27.72%, 32.48%, and 27.51%) for AA073, AA212, and AA128, respectively.

Rice straw was harvested in the vegetative stage before seed formation occurred. After drying in the sun, rice straw was packaged into three 1-ton bags and stored in a ventilated and rodent-free storage room until use. Roasted *Morama* bean cake and sunflower seed cake were processed as indicated in experiments one and two. During processing, the feeds (rice straw and oilseed cake) were ground in a laboratory mill (Willy Mill; Thomas Willey Laboratory Mill, Philadelphia, USA) using 1 mm mesh and 2 mm sieves for chemical analysis and *in vitro* incubations, respectively

Approximately 500 g of each byproduct derived from the processing and/or extraction of vegetable oil was collected. Subsequently, samples were transported to the laboratory, packaged, and stored at 4°C prior to analysis. In the laboratory, the samples belonging to each oilseed press cake or rice straw were thoroughly homogenized. Different mixtures based on roasted *Morama* bean cake (RMBC) and sunflower seed cake (WSSC) as protein sources, with rice straw (RS) as roughage (Table 4.1), were created. The feed combinations resulted in a total of six diets. The dietary treatments were divided into two subgroups. The first group was based on three levels of inclusion of roasted *Morama* bean cake mixed with rice straw. The second

group was based on sunflower seed cakes in three different inclusion rates mixed with rice straw. Each diet was formulated to evaluate various levels of cakes, with ratios of 100/0, 90/10, 80/20, and 70/30% for each oilseed press cake mixed with rice straw (rice straw/oilseed cake).

4.2.4 Analysis of fatty acids from sunflower and Morama

A 0.5 kg package of each oilseed (sunflower and roasted *Morama*) was taken to the National Food Research Technology Center (NFRTC) for fatty acid profiling. Samples were extracted in triplicate using a modified method of AOAC (1996). Briefly, samples were directly converted to fatty acid methyl ester (FAME) and analyzed by capillary gas chromatography. An HP 5890 Series II gas chromatograph (Hewlett Packard Co., Palo Alto, CA) equipped with a quartz glass capillary column (DB23 60 m 0.25 mm, 0.25 μ m d.f.) was used.

To quantify the fatty acid content of the samples (in percent by weight), synthetic fatty acids (typically C13:0, C19:0, C21:0, or C23:0) were added to the samples as internal standards before extraction. The methyl esters were then quantified using ChemStation software (Agilent Technologies, Wilmington, DE). Helium was used as the carrier gas, the temperature of the flame ionization detector was set to 260°C, and the split ratio was 80:1. The temperature was initially set at 140°C for three minutes, then increased to 240°C for one minute and held for 10 minutes, and finally lowered to 140°C and held for a fraction of a minute

4.2.5 Feed diets preparation for chemical analysis

The chemical composition of diets (rice straw + oilseed cake) was run in triplicate. The dry matter content of the diets was measured by drying samples in an air-forced oven at 60°C for 48 hours, and ash content was measured by placing samples into a muffle furnace at 550°C for 5 hours. Nitrogen (N) was measured by the Kjeldahl method using selenium (Se) as a catalyst, and crude protein (CP) was calculated as 6.25 x N. Ether extract (EE) was measured by weight

loss of the dry matter on extraction with diethyl ether in an ANKOM 220 Fibre Analyzer unit (ANKOM Technology Corporation, Fairport, NY, USA) according to the Association of Analytical Chemists (AOAC) methods (AOAC, 2005).

The neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, and hemicellulose were evaluated following the procedures by Van Soest (1973) and Van Soest *et al.* (1991). Sodium sulphite and heat-stable amylase (17,400 Liquefon units/mL, ANKOM) were used in NDF determination. ADL was determined by treating ADF residue with 72% sulphuric acid.

4.2.6 Animal and preparation of rumen inoculum

The rumen fluid used was obtained from a mature Tswana ox, weighing approximately 820 kg, and fitted with a permanent rumen cannula. The animal received a daily ration of rice straw and 0.25 kg of commercially available compound feed (120 g/kg crude protein) for ten days. Additionally, the animal was provided with a lick made of dicalcium phosphate and coarse salt at ratio of 1:2. The feed composition covered the energy and protein requirements for maintenance and balanced the energy-protein ratio in the rumen. The ox was kept in accordance with veterinary animal welfare regulations.

Collection of rumen content took place in the morning after the animal had fasted since the last feeding. Just over 1000 ml of rumen fluid was collected from the ox in two pre-warmed thermos flasks and sent to the laboratory (BUAN). The pH of the rumen fluid was checked at the time of collection and at regular intervals between *in vitro* degradation phases.

4.2.7 PH measurement of the rumen fluid

The rumen fluid and supernatant aspirated from the *in vitro* digestion vessels were measured using a laboratory pH meter (HANNA 8520 Instruments QA, Supplies, LLC.1185 Pineridge Road Norfolk, Virginia, USA). The pH meter probes were dipped into the rumen fluid/supernatant three times and each time the reading was recorded and averaged to form the final pH value.

4.2.8 Preparation for *in vitro* Fermentations

The flowchart in Figure 4.1 illustrates the Tilly and Terry (1963) method, subsequently modified by Robinson *et al.* (1999), which was used in this study to determine feed degradabilities, rumen fermentation characteristics, and post-rumen digestibilities. Seven feed treatments were analyzed using the ANKOM *in vitro* fermentation system Daisy^{II} (ANKOM Technology Corp., Fairport, NY, USA). Clean, dry centrifuge tubes (50 mL, Polypropylene (PP), a Thomas Scientific Brand, Germany) were rinsed in acetone for five minutes before adding the ground rice forage.

After measuring the weight of the tubes, a mixture of milled rice straw and oilseed cake was added to each tube to achieve a total weight of 2.0 g. The proportions of rice straw and oilseed cake varied based on the inclusion levels of 0%, 10%, 20%, and 30%.

After the rumen fluid was delivered to the laboratory, it was filtered through four layers of cheesecloth and diluted in the buffer medium at a ratio of 1:4 (v/v) according to the ANKOM manual (ANKOM Technology), and the pH was adjusted to 6.8. Each treatment was replicated eight times, resulting in 56 sample tubes plus four blanks (a total of 60 tubes) in incubation runs (Figure 5.0). Then, 20 mL of buffered rumen fluid was dispensed into each of the tubes under a carbon dioxide purge and closed with plastic lids fitted with 4 mm tubing (reinforced

with Bostik to avoid air leakage) that led to the outside of the chamber to ease the buildup of gases. The centrifuge tubes with feed samples were placed in the rotating incubators at $39\pm0.5^{\circ}\text{C}$ for 48 hours (Stage 1). At the end of the incubation, microbial activity was halted by immersing the tubes in ice water. Half of the tubes ($n = 28$) were then opened, and the pH readings were measured and recorded. The supernatant in the tubes was decanted, and clean tap water was added for rinsing until the water ran clear. *In vitro* apparent dry matter degradability (*ivADMdeg*) was determined according to Minson and McLeod (1980), where the dry matter degradability is calculated by comparing the initial weight of the feed sample to the weight of the undigested residues. Neutral detergent fibre (NDF) for the determination of truly degraded dry matter was determined according to Goering and Van Soest (1970). The feed mixtures were analyzed for *in vitro* apparent crude protein degradability (*ivACPdeg*) and computed as the difference between the CPs of the feed sample minus the by-pass crude protein ($\text{CP} - \text{BP} - \text{CP}$). The bypass crude protein is calculated by comparing the nitrogen content of the undigested residues to the initial nitrogen content of the feed sample. The formula used is:

$$\text{BP} - \text{CP} (\%) = \left(\frac{\text{nitrogen in undigested residues}}{\text{initial nitrogen in feed sample}} \right) \times 100$$

The remaining tubes ($n = 28$) were also opened, infused with 6 mL of 20% HCl and 2 mL of 5% pepsin (Pepsin P-700, Sigma-Aldrich, St. Louis, MO, USA) solution (Wulandari *et al.*, 2020), also a modification of Tilley and Terry (1963). Neutralization of the pepsin-HCl reaction was carried out according to the method of Serrepica *et al.* (2019). After 60 minutes of incubation, 2 mL of NaOH was added to neutralize the medium and stop the pepsin reaction. This was followed by the addition of a buffered enzymatic mixture (1.8 M KH_2PO_4), which contained trypsin (20 mg/mL; Trypsin T4799, Sigma-Aldrich), chymotrypsin (20 mg/mL;

alpha-chymotrypsin type II, C4129, Sigma-Aldrich), amylase (50 mg/L; alpha-amylase type I-A, A6255, Sigma-Aldrich), and lipase (4 mg/mL; Lipase type VI-S, L0382, Sigma-Aldrich).

The tubes were left for 24 hours in the Daisy^{II} incubator. At the end of the incubation, the residues were rinsed in cold tap water until the water was clear. The residues were then dried at 60°C for 48 hours and weighed. *In vitro* apparent dry matter digestibility (ivADMdig) was calculated by the weight difference between the incubated matter and the original weight. *In vitro* apparent crude protein digestibility (ivACPdig), or intestinal crude protein digestibility, was determined by multiplying bypass crude protein (BP-CP) by bypass crude protein digestibility (BP-CPdig). The bypass crude protein (BP-CP) or rumen undegradable crude protein was computed as the ratio between the recovered undegraded crude protein (CP) after the ruminal phase and the corresponding CP of the feed material, according to the method of Serrepica *et al.* (2019). *In vitro* truecrude protein digested (ivTCPdig) comprised the CP rumen degradable and post-ruminal digested crude protein (sum of ivACPdeg and ivACPdig). In each case, parameter determination was performed after treatment variables (degradability or digestibility) were pooled from replicates.

4.2.9 Volatile fatty acid analysis from feed treatments

The supernatant from different feed inoculants was collected after 12 hours and sent to the Botswana Institute of Research and Innovation (BITRI) for volatile fatty acid (VFA) analysis. Volatile fatty acid (VFA) analysis was performed by pooling half of the replicate contents for each treatment, resulting in a total of 14 treatments. Briefly, 6 mL of the pooled fluid contents were transferred into 10-mL plastic tubes and centrifuged at 15,000 x g for 15 minutes. Then, 3 mL of the supernatant was aspirated and centrifuged at 15,000 x g for 15 minutes. After centrifugation, a 0.8-mL aliquot of the supernatant was added into an auto-sampler vial with

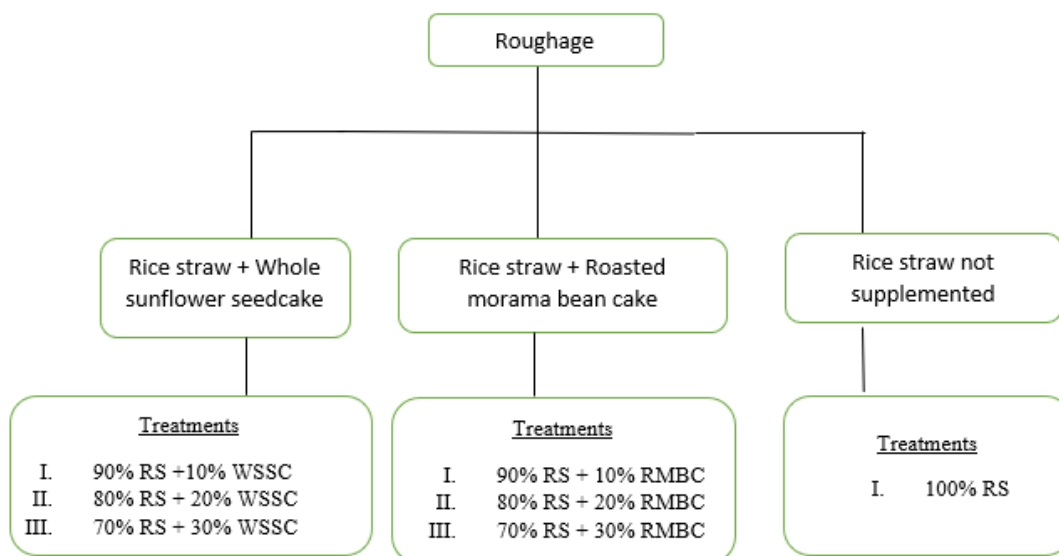
0.9 mL of internal standard (0.75 mM 3-methyl valeric acid). Volatile fatty acid concentrations were determined using capillary gas chromatography (SCION Instruments NL BV, Amundsenweg 22–24, 4462 GP Goes, The Netherlands) according to Getachew *et al.* (2005).

4.2.10 Ammonia determination in rumen fluid

A portion (500 mL) of rumen fluid (directly from the animal) was acidified with 25 μ L of hydrochloric acid and refrigerated at -20°C until analysis, while the other portion was reserved for degradation studies. During the incubation of samples, ammonia determination was performed according to De Waal (1986). Briefly, rumen fluid was either thawed or removed from incubation containers and 20 mL was centrifuged at 15,000 x g for 15 minutes. For each treatment, the supernatant was aspirated into centrifuge tubes in triplicate and added to labeled distillation tubes using a 20-mL pipette. Approximately 20 g of magnesium oxide (MgO), 0.4 mL of octanol (C₈H₁₈O), and 50 mL of distilled water were added to the distillation unit. The distillation tube was attached to a Becchi distillation unit, and the distillation was completed in 5 minutes. A dispenser was also connected to the distillation unit, into which a mixture of MgO, octanol, and water in a 2% boric acid plus indicator solution was dripped. The indicator changed its color from purple to green. Finally, the distillate was titrated to the original colour (purple) with 0.01 N H₂SO₄; this quantified the amount of H₂SO₄ that resulted in the colour change. The formula shown below was used to estimate ammonia concentration:

$$[\text{NH}_3] = \frac{(0.14 \times N \text{ of H}_2\text{SO}_4) \times (\text{NO. of titrations} \times 1.2143)}{\text{sample size (ml)}} \times 100\% \quad \text{Equation 1}$$

Where, [NH₃] – concentration of ammonia (mg NH₃ per 100 ml); N H₂SO₄ – normality of sulphuric acid; 0.14 – constant; 1.2143 – constant.



Where, RS - rice straw without supplements; WSSC+RS – whole sunflower seedcake and rice straw; RMBC+roasted morama bean cake –roasted morama bean cake

Figure 4.1: Schematic diagram illustrating dietary treatments evaluated in *in vitro* 2-stage experiment (Tilly and Terry, 1963)

4.2.11 Experimental design

Rice straw and oilseed cakes (RMBC and WSSC) were mixed by binary combination as shown below:

1. Rice straw without supplementation 100% (RS100%)
2. Rice straw (90%) + roasted *Morama* bean cake (10%) (90%RS + 10%RMBC)
3. Rice straw (80%) + roasted *Morama* bean cake (20%) (80%RS + 20%RMBC)
4. Rice straw (70%) + roasted *Morama* bean cake (30%) (70%RS + 30%RMBC)
5. Rice straw (90%) + whole sunflower seedcake (10%) (90%RS + 10%WSSC)
6. Rice straw (80%) + whole sunflower seedcake (20%) (80%RS + 20%WSSC)
7. Rice straw (70%) + whole sunflower seedcake (30%) (70%RS + 30%WSSC)

The substrate formulation described above was subjected to a *t*-test or independent comparison to evaluate differences between RMBC and WSSC at identical supplementation levels (0%,

10%, 20%, and 30%). The experimental setup followed a completely randomized design (CRD) matrix, with three replicates per treatment combination.

4.2.12 Statistical analysis

The experimental data, including chemical composition, degradability, and digestibility parameters, were analyzed using a statistical model tailored to the experimental design. Each oilseed cake type (roasted *Morama* bean cake [RMBC] and whole sunflower seed cake [WSSC]) was analyzed separately to evaluate the effects of supplementation levels (0%, 10%, 20%, 30%). A One-Way Analysis of Variance (ANOVA) was performed within a completely randomized design (CRD) for each oilseed cake type to assess the effect of supplementation levels on the response variables. Where significant differences were identified, post-hoc pairwise comparisons were conducted using Tukey's HSD test to determine specific group differences. Statistical analyses were implemented using the general linear model (GLM) procedure of the SAS software package (version 9.2; SAS Institute, Cary, NC, USA, 2012). The data were therefore analysed according to the model:

$$T = \frac{x_1 - x_2}{\sqrt{(s_1^2/n_1 + s_2^2/n_2)}}$$

where, **X₁ and X₂**: means of the two groups being compared (the mean response variable for RMBC and WSSC at a specific supplementation level); **s₁² and s₂²**: The variances of the two groups; **n₁ and n₂**: The sample sizes of the two groups (number of replicates for RMBC and WSSC, respectively); **T**: The calculated t-statistic, which measures the difference between the group means relative to the variability within each group.

Ammonia (NH₃) and volatile fatty acid concentrations were obtained from similar pooled samples at 12 hours of incubation. Control treatments (RS; rice straw) and rice straw supplemented with oilseed cakes (roasted *Morama* bean cake and whole sunflower seedcake)

were compared using a set of pre-planned orthogonal contrasts, with their coefficients for respective treatments. Orthogonal contrasts were made for specific comparisons between treatments as shown:

- Compare the control treatment (RS100%) with all supplemented treatments.
- Compare RMBC treatments with WSSC treatments.
- Compare different levels of RMBC.
- Compare different levels of WSSC.

Generally, *in vitro* ruminal degradability and post-ruminal digestibility of rice straw with or without supplements were analyzed to estimate how different chemical components (such as proteins, fibres, and carbohydrates) of the rice straw are broken down and absorbed in the digestive system.

Significant differences were identified at a P-value of less than 0.05, unless specified otherwise.

4.3 Results

4.3.1 Chemical composition

The chemical composition of basal diets with or without oilseed press cakes is shown in Table 4.1. The study results show that the chemical composition of rice straw with or without supplements was significantly different ($P < 0.05$) for DM, CP, EE, ADF, NDF, ADL, cellulose, hemicellulose, and non-fiber carbohydrates. The DM of the feed treatments ranged from 92.6% (RS + 10% RMBC) to 94.4% (RS + 30% WSSC). The highest CP (19.7%) was noted for RS + 30% RMBC ($P < 0.0001$). The highest ($P < 0.050$) ether extract (EE) was detected in rice straw mixed with 30% roasted *Morama* bean cake compared to any of the other feed treatments. Non-fiber carbohydrates (NFC) were lowest in rice straw without supplements (1.2%; $P = 0.023$) compared to all six feed treatments. The rice straw alone had the highest ADF, NDF, ADL, and ash contents of 35.2%, 46.3%, 8.6%, and 10.8%, respectively.

Furthermore, the hemicellulose and cellulose content of rice straw varied with or without oilseed press cake (10.1% and 24.6%, respectively). Some chemical components, such as organic matter and ash, were not affected by oilseed cake supplementation ($P = 0.9886$).

Table 4.1: Chemical composition of rice straw, interaction of rice straw mixed with roasted morama bean cake and interaction of rice straw mixed with whole sunflower seed cake at different concentration (0, 10, 20 and 30%)

Index	DM basis							P-value	
	RS	RMBC+RS				WSSC+RS			
	0	10	20	30	10	20	30		
DM	94.0±0.5 ^a	91.2±0.2 ^b	90.5±1.2 ^{ab}	89.7±0.2 ^{ab}	92.3±0.2 ^{ab}	92.6±0.1 ^{ab}	91.2±0.1 ^a	0.053*	
OM	83.2±1.0	81.1±1.3	80.4±1.3	79.6±1.5	81.4±0.1	82.5±0.1	80.5±1.0	0.9888	
Ash	10.8±0.5 ^a	10.1±1.3	10.1±0.1	10.1±3.6	10.9±0.1	10.1±0.1	10.7±3.8	0.9884	
CP	5.24±0.1 ^g	8.97±0.1 ^c	10.4±0.1 ^c	19.7±0.04 ^a	7.83±0.04 ^f	9.8±0.1 ^d	11.5±0.3 ^b	<.0001*	
EE	1.3±0.6 ^d	6.3±0.3 ^{bc}	6.3±0.6 ^a	6.9±1.3 ^{ab}	4.8±0.9 ^c	6.0±0.8 ^{bc}	5.6±0.5 ^c	0.050*	
ADF	35.2±0.8 ^a	24.7±0.4 ^c	23.9±0.3 ^c	22.1±0.9 ^c	26.5±0.8 ^b	25.1±0.5 ^b	23.8±0.5 ^b	<.0001*	
NDF	46.3±3.7 ^a	44.2±0.9 ^a	42±2.8 ^b	34.3±4.1 ^b	44.9±0.8 ^a	44.1±0.3 ^a	42±3.0 ^a	0.0027*	
ADL	8.6±1.4 ^a	7.1±2.8 ^b	6.4±0.7 ^c	8.1±0.6 ^b	6.5±1.1 ^c	7.4±0.2 ^b	6.9±0.8 ^b	0.0116*	
Cellulo.	24.6±0.6 ^b	18.5±3.1	18.9±0.5	16.9±0.3	27.0±1.2 ^a	24.9±0.6 ^b	25.9±0.9 ^a	0.0009*	
Hemice.	10.1±2.9 ^b	22.6±1.2 ^a	15.5±2.5 ^{ab}	10.3±4.1 ^b	18.4±0.3 ^a	17.8±0.2 ^{ab}	15.7±3.5 ^{ab}	0.0075*	
NFC	1.20±0.2 ^d	5.70±0.2 ^b	6.6±0.01 ^{ab}	7.5±2.1 ^a	2.6±0.3 ^c	4.3±0.4 ^c	6.4±0.1 ^{ab}	0.023*	

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; ADF- acid detergent fibre; NDF- neutral detergent fibre; ADL- acid detergent lignin; RS – rice straw without supplements; RMBC+RS- diet with a combination of roasted morama bean cake and rice straw; WSSC+RS – diet with a combination of whole sunflower seedcake and rice straw; * indicates $P < 0.05$ means significantly different; ^{a, b, c, d} means with different scripts within the row are significant at $P < 0.05$

4.3.2 Ruminal Fermentation characteristics of feeds

Fermentation characteristics of rice straw with or without supplements are presented in Table 4.2. The highest pH values were observed in rice straw without supplements and rice straw mixed with oilseed cake with the inclusion of 30% WSSC, followed by 20% WSSC, 10% WSSC, 10% RMBC, 30% RMBC, and 20% RMBC ($P < 0.05$) in that order.

Rice straw with 30% RMBC showed the highest ammonia concentration, followed by the inclusion of 20% RMBC, 30% WSSC, 10% RMBC, 20% WSSC, 10% WSSC, and rice straw without supplements (Table 4.2; $P < 0.0001$).

The total volatile fatty acids showed divergent distributions of acetate, propionate, and butyrate. The molar proportions of acetate and propionate differed significantly ($P < 0.0001$) among the levels of the oilseed press cakes. The highest ($P < 0.0001$) acetate values are presented in the order of 70% RS + 30% RMBC, 80% RS + 20% RMBC, 70% RS + 30% WSSC, 80% RS + 20% WSSC, 90% RS + 10% RMBC, and 90% RS + 10% WSSC. Rice straw without supplements had low acetate production. The supplemental inclusion levels of 30% for both RMBC and WSSC had higher (Table 5.2; $P < 0.0001$) acetate production than any other feed treatments. However, propionate concentrations for the rice straw supplemented with oilseed cakes uniformly decreased ($P < 0.05$), except with a 30% RMBC inclusion level, which showed an increase in propionate production ($P < 0.0001$).

The production of butyrate from rice straw supplemented with protein supplements was stable at all levels and did not differ ($P > 0.05$). A comparison of cake substitution levels showed that the 70% RS + 30% RMBC inclusion had the lowest acetate: propionate ratio, whereas the remaining oilseed cake levels were slightly higher and did not differ in acetate-to-propionate ratio ($P > 0.05$).

Table 4.2: Mean values, probability (p-value), and standard error of the mean (SEM) of pH and production of total volatile fatty acids [acetate (mmol/L), propionate (mmol/L), and butyrate (mmol/L)] after supplementation of rice straw with oilseed press cakes at different levels after 48 hours of *in vitro* incubation

OILSEED CAKE	LEVELS OF SUPPLEMENTATION				SEM
	0%	10%	20%	30%	
	pH	pH	pH	pH	
RMBC	6.93	6.50	6.43	6.45	0.120

WSSC	6.93	6.52	6.53	6.55	0.099
P-VALUE	0.934	0.955	0.890	0.933	
SEM	0.001	0.001	0.033	0.033	
Production of ammonia (NH₃)					
RMBC	4.51	8.20 ^a	10.41 ^a	15.32 ^a	2.259
WSSC	4.51	6.92 ^b	7.51 ^b	9.20 ^b	0.970
P-VALUE	0.890	0.054	0.043	0.035	
SEM	0.003	0.427	0.967	2.040	
Total volatile fatty acid (μmol/mL)					
RMBC	8.22	13.98 ^a	17.65 ^a	22.52 ^a	3.016
WSSC	8.22	11.35 ^b	14.92 ^b	17.78 ^b	2.083
P-VALUE	0.912	0.045	0.036	0.026	
SEM	0.003	0.878	0.912	1.578	
Production of acetate (μmol/mL)					
RMBC	6.11	10.20 ^a	12.91 ^a	16.22 ^a	2.140
WSSC	6.11	8.33 ^b	11.00 ^b	13.12 ^b	1.531
P-VALUE	0.921	0.046	0.054	0.047	
SEM	0.003	0.625	0.621	1.032	
Production of propionate (μmol/mL)					
RMBC	1.53	3.38	4.34	6.02 ^a	0.938
WSSC	1.53	2.69	3.57	4.32 ^b	0.600
P-VALUE	0.981	0.067	0.061	0.044	
SEM	0.003	0.232	0.257	0.567	
Production of butyrate (μmol/mL)					
RMBC	0.59	0.40	0.41	0.30	0.060
WSSC	0.59	0.36	0.35	0.36	0.058
P-VALUE	0.092	0.063	0.064	0.075	
SEM	0.003	0.013	0.020	0.020	
Acetate: propionate ratio					
RMBC	3.99	3.02	2.97	2.69	0.284
WSSC	3.99	3.09	3.08	3.03	0.231
P-VALUE	0.087	0.067	0.068	0.088	
SEM	0.003	0.023	0.037	0.113	

RMBC – roasted morama bean cake; WSSC – whole sunflower seedcake; *means within the same column with different letters differ statistically by Tukey's test at $P < 0.05$ significance level.

Furthermore, Figures 4.2, 4.3, and 4.4 show regressions of *in vitro* dry matter degradability (*ivADMdeg*), *in vitro* truly degradable dry matter (*ivTDMdeg*), *in vitro* organic matter degradability (*ivOMdeg*), and total volatile fatty acids during *in vitro* ruminal feed fermentations, which were not significant ($P > 0.05$).

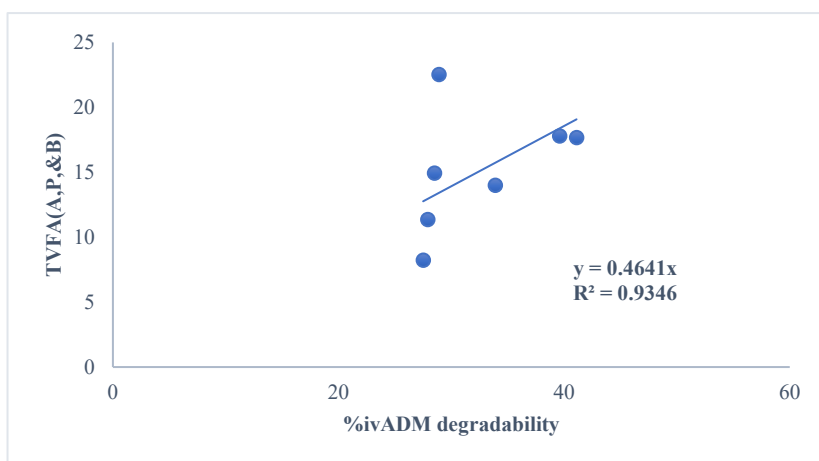


Figure 4.1: Regression of total volatile fatty acid production on in vitro truly degradable dry matter (*ivTDMdeg*)

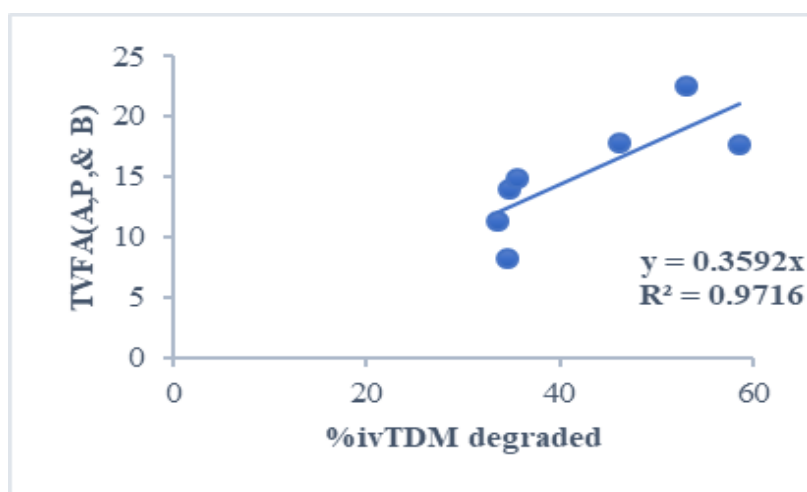


Figure 4.2: Regression of total volatile fatty acid production on *in vitro* truly degradable dry matter (*ivTDMdeg*)

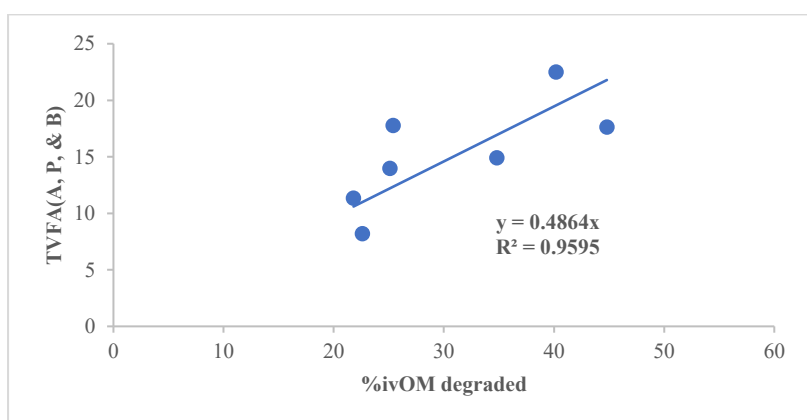


Figure 4.3: Regression of total volatile fatty acid production on *in vitro* organic matter degradability(*ivOMdeg*)

4.3.3 *In vitro* ruminal dry matter degradability and post rumen dry matter digestibility

The effects of rice straw without supplements and rice straw supplemented with either roasted *Morama* bean cake or whole sunflower seed cake on *in vitro* true dry matter degradability (*ivTDMdeg*), organic matter degradability (*ivOMdeg*), *in vitro* apparent dry matter degradability (*ivADMdeg*), and *in vitro* apparent dry matter digestibility (*ivADMdig*) are shown in Table 4.3 and Figures 4.5, 4.6, 4.7, and 4.8. There were variable interactions between rice straw and oilseed cake type at different inclusion rates on *ivTDMdeg*, *ivOMdeg*, and *ivADMdeg* over a 48-hour period. The highest ($P < 0.0001$) *in vitro* apparent dry matter degradability (*ivADMdeg*) was observed in the following order: 20% RMBC + 80% RS, 30% WSSC + 70% RS, 10% RMBC + 90% RS, 30% RMBC + 70% RS, 20% WSSC + 80% RS, 10% WSSC + 90% RS, and 100% RS.

As for the level of *in vitro* truly degradable dry matter (*ivTDMdeg*) of the rice straw with or without supplements, the trend was as follows: 20% RMBC + 80% RS, 30% RMBC + 70% RS, 30% WSSC + 70% RS, 20% WSSC + 80% RS, 10% RMBC + 90% RS, 10% WSSC + 90% RS, and 100% RS (without supplements).

The level of *in vitro* organic matter degradability (*ivOMdeg*) of rice straw mixed with oilseed cakes also showed a similar trend, with the order of magnitude as follows: 20% RMBC + 80% RS, 30% RMBC + 70% RS, 20% WSSC + 80% RS, 30% WSSC + 70% RS, 10% RMBC + 90% RS, 10% WSSC + 90% RS, and 100% RS (without supplements).

The magnitude of post-ruminal dry matter digestibility (*ivADMdig*) of rice straw with or without supplements was in the following order: 30% RMBC + 70% RS, 20% RMBC + 80% RS, 30% WSSC + 70% RS, 10% RMBC + 90% RS, 10% WSSC + 90% RS, and 100% RS (without supplements). The mean proportion of abomasal *in vitro* dry matter digestibility of

rice straw supplemented with roasted *Morama* bean cake was higher than that of rice straw supplemented with whole sunflower seedcake, but the difference was not statistically significant (56.5% vs. 50.5%; $P>0.05$).

In all cases, the *in vitro* ruminal degradability and abomasal digestibility of rice straw without supplements were very low (Figures 4.5, 4.6, 4.7, and 4.8; $P < 0.05$).

Table 4.3: Mean values, probability (P-value), and standard error of the mean (SEM) of *in vitro* apparent dry matter degradability (ivADMdeg, %), *in vitro* true dry matter degradability (ivTDMdeg, %), *in vitro* organic matter degradability (ivOMdeg, %), and *in vitro* apparent dry matter digestibility (ivADMdig, %) of rice straw supplemented with *Morama* and sunflower cakes at different levels after 48 hours of ruminal *in vitro* incubation.

Oilseed cake	Levels of oilseed press cake inclusion rates				RMSE
	0%	10%	20%	30%	
<i>in vitro</i> apparent dry matter degradability(<i>iv</i>ADMdeg)					
RMBC	27.5	33.9 ^a	41.1 ^a	28.9 ^b	3.074
WSSC	27.5	27.9 ^b	28.5 ^b	39.6 ^a	2.916
P-value	0.90	0.057	0.043	0.051	
SEM	0.003	1.999	4.212	3.550	
<i>in vitro</i> truly degradable dry matter(<i>iv</i>TDMdeg)					
RMBC	34.6	34.8	58.5 ^a	53.1 ^a	6.190
WSSC	34.6	33.6	35.5 ^b	46.2 ^b	2.934
P-value	0.90	0.085	0.028	0.046	
SEM	0.003	0.418	7.668	2.210	
<i>in vitro</i> organic matter degradability(<i>iv</i>OMdeg)					
RMBC	22.6	25.1 ^a	44.8 ^a	40.2 ^a	5.489
WSSC	22.6	21.8 ^b	34.8 ^b	25.4 ^b	2.985
P-value	0.901	0.042	0.004	<0.001	
SEM	0.003	1.100	3.333	4.933	
<i>in vitro</i> apparent dry matter digestibility(<i>iv</i>ADMdig)					

RMBC	42.7	49.9 ^a	55.3	64.4 ^a	4.573
WSSC	42.7	44.5 ^b	55.7	51.3 ^b	3.018
P-value	0.901	0.049	0.087	0.039	
SEM	0.003	1.800	0.133	4.367	

RMBC – roasted morama bean cake; WSSC – whole sunflower seedcake; means in the same column with different letters differ statistically by Tukey's test at $P < 0.05$.

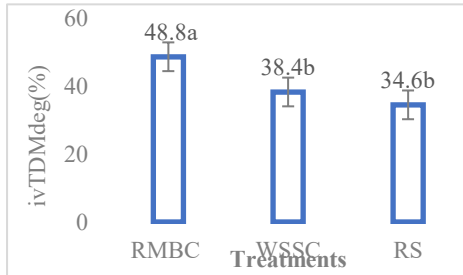


Figure 4.4: The mean percentage of *in vitro* true dry matter degradability (ivTDMdeg) for each group of treatments

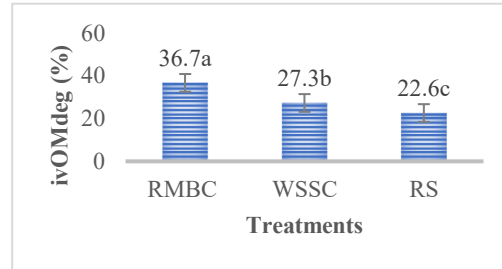


Figure 4.5: The mean percentage of *in vitro* organic matter degradability (ivOMdeg) for each group of treatments

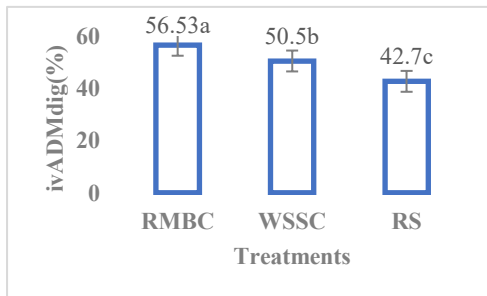


Figure 4.6: The mean percentage of *in vitro* apparent dry matter digestibility (ivADMdig) for each group of treatments

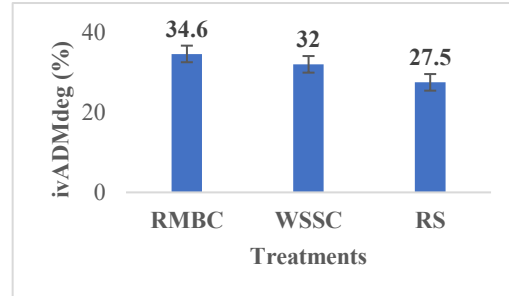


Figure 4.7: The mean percentage of *in vitro* apparent dry matter degradability (ivADMdeg) for each group of treatments

4.3.4 *In vitro* ruminal crude protein degradability and post-ruminal crude protein digestibility

The effects of rice straw with or without oilseed press cakes on *in vitro* apparent crude protein degradability (ivACPdeg), *in vitro* apparent crude protein digestibility (ivACPdig), available bypass crude protein (BP-CP), and bypass crude protein digestibility (BP-CPdig) are shown in

Table 4.4 and Figures 4.9, 4.10, 4.11, 4.12, and 4.13. The *ivACPdeg*, *ivACPdig*, BP-CP, and BP-CPdig of rice straw supplemented with either roasted *Morama* bean cake or whole sunflower seed cake were significantly higher ($P < 0.05$) than those of rice straw without supplements.

The proportion of *in vitro* apparent crude protein degradability (*ivACPdeg*) of rice straw with or without supplements occurred in the following descending order: 30% WSSC, 20% RMBC, 30% RMBC, 10% WSSC, whereas 10% RMBC and 20% WSSC did not differ ($P > 0.05$), and RS. The *ivACPdeg* of rice straw supplemented with roasted *Morama* bean cake increased linearly from a 10% inclusion rate to 20% by 4.8 units; it dropped when the inclusion rate was raised to 30% (Table 4.4). In contrast, the addition of 20% whole sunflower seed cake resulted in a reduction in *ivACPdeg* of the feed treatment, whereas the addition of 30% WSSC increased the protein degradability (Table 4.4). The mean *ivACPdeg* of roasted *Morama* bean cake in various combinations with rice straw was slightly higher than that of the whole sunflower seed cake, but the difference was not statistically significant (59.7% vs. 58.8%; $P > 0.05$).

The following order of inclusion rates: 20% WSSC, 10% RMBC, 10% WSSC, 30% RMBC, 20% RMBC, 30% WSSC, and RS reflected the order of available bypass crude protein (BP-CP) of rice straw with or without supplements. The BP-CP increased with the addition of the oilseed cake supplements, except for the inclusion of 30% WSSC, which decreased by 9.3%. There was no significant mean increase in BP-CP content of rice straw mixed with whole sunflower seed cake compared to rice straw mixed with roasted *Morama* bean cake (41.2% vs. 40.3%; $P > 0.05$).

The bypass crude protein digestibility (BP-CPdig) of the feed treatments with or without supplements showed a descending order based on oilseed inclusion rates as follows: 10%

RMBC, 20% RMBC, 10% WSSC, 20% WSSC, 30% WSSC, 30% RMBC, and RS. There was an increase in bypass crude protein digestibility after supplementing rice straw with 30% RMBC. The numerically highest (Figure 4.11) mean value of bypass crude protein digestibility was found in rice straw supplemented with roasted *Morama* bean cake compared to whole sunflower seed cake (31.6% vs. 28.3%).

The decrease in *in vitro* apparent crude protein digestibility (*ivACPdig*) of rice straw supplemented with RMBC or WSSC occurred at the following inclusion rates in descending order: 30% RMBC, 30% WSSC, 20% RMBC, 10% RMBC, 20% WSSC, 10% WSSC, and RS. The *ivACPdig* showed linear responses ($P < 0.0001$) to the inclusion of both oilseed cakes. The numerically highest, although not statistically significant, mean value was found when rice straw was mixed with roasted *Morama* bean cake compared to when rice straw was mixed with whole sunflower seed cake (Figure 4.12).

In vitro total crude protein digested (*ivTCPdig*; total tract digested protein) from rice straw supplemented with oilseed cakes reflected the inclusion of oilseed cakes in descending order: 30% WSSC, 30% RMBC, 20% RMBC, 10% RMBC, 10% WSSC, 20% WSSC, and RS. *IvTCPdig* showed linear responses ($P < 0.0001$) based on the oilseed cake inclusion rate, except for the addition of 20% WSSC where it decreased by 2% before increasing by 7.2%. RMBC showed the numerically highest (Figure 4.15; $P > 0.05$) mean of *ivTCPdig* compared to the other supplement (WSSC; 37.2% vs. 36.2%).

Table 4.4: Mean values, probability (P-value), and standard error of the mean (SEM) of *in vitro* apparent crude protein degradability (%), bypass crude protein (g/kg), bypass crude protein digestibility (%), *in vitro* crude protein digestibility (%), and total crude protein utilization (%) in rice straw supplemented with roasted *Morama* bean and whole sunflower seed cakes at different levels.

Oilseed cake	Levels of substitution	SEM
--------------	------------------------	-----

	0%	10%	20%	30%	
<u>ivACPdeg</u>					
RMBC	22.9	56.9	61.7 ^a	60.4	9.247
WSSC	22.9	58.7	54.2 ^b	63.5	9.174
P-value	0.095	0.066	0.038	0.062	
SEM	0.003	0.900	2.500	1.033	
<u>BP-CP</u>					
RMBC	19.8	43.1	38.3 ^b	39.6 ^a	5.232
WSSC	19.8	41.3	45.8 ^a	36.5 ^b	5.677
P-value	0.094	0.092	0.053	0.054	
SEM	0.003	0.600	2.500	1.017	
<u>BP-CPdig</u>					
RMBC	4.8	34.6 ^a	32.7 ^a	36.4	6.863
WSSC	4.8	29.4 ^b	27.6 ^b	27.8	6.88
P-value	0.089	0.052	0.053	0.089	
SEM	0.003	1.733	1.700	0.133	
<u>ivACPdig</u>					
RMBC	0.95	14.9 ^a	15.5	21.1 ^a	4.288
WSSC	0.95	12.1 ^b	12.6	17.7 ^b	3.530
P-value	0.091	0.053	0.061	0.032	
SEM	0.003	0.933	0.967	1.133	
<u>ivTCPdig</u>					
RMBC	11.9	35.9	37.1 ^a	38.5	6.339
WSSC	11.9	35.4	33.4 ^b	40.6	6.326
P-value	0.089	0.088	0.052	0.067	
SEM	0.003	0.167	1.233	0.700	

RMBC – roasted morama bean cake; WSSC – whole sunflower seedcake; *ivACPdeg* – *in vitro* apparent crude protein degradability; BP-CP – bypass crude protein; BP-CPdig- bypass crude protein digestibility, *ivACPdig* – *in vitro* apparent crude protein digestibility; *ivTCPdig* – *in vitro* total crude protein digestibility; the means in column with different superscripts differ ($P<0.05$); SEM – standard error of mean.

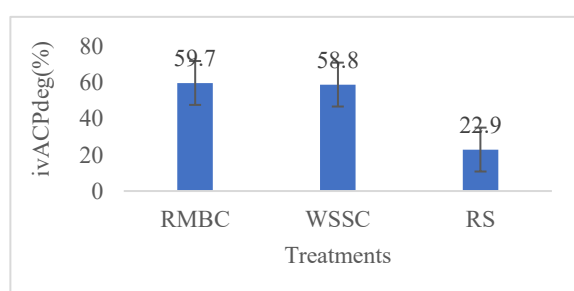


Figure 4.8: The mean of *in vitro* apparent crude protein degradability (*ivACPdeg*) for each group treatment

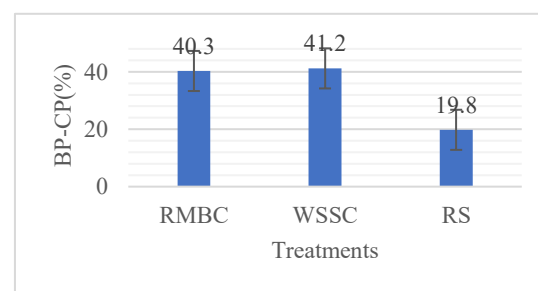


Figure 4.9: The mean bypass crude protein (BP-CP) for each the group treatment

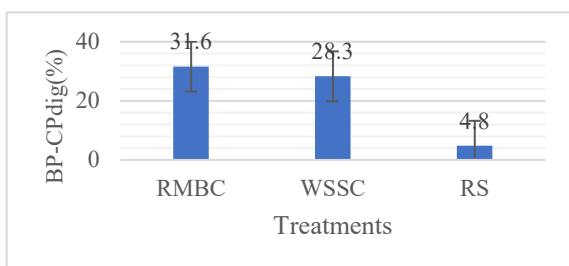


Figure 4.10: The mean of bypass crude protein digestibility (BP-CPdig) for each group treatment

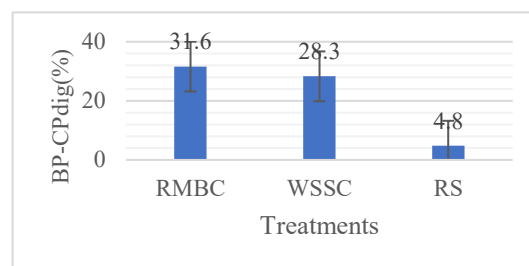


Figure 4.11: The mean of in vitro apparent crude protein digestibility (ivACPdig) for each group treatment

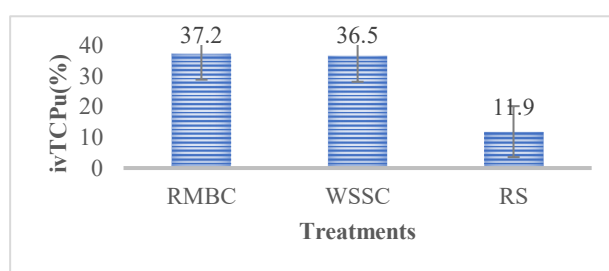


Figure 4.12: The mean of in vitro total crude protein digestibility (ivTCPdig) for each group treatment.

4.5 Discussion

4.5.1 Chemical analysis

The mean chemical composition of rice straw combined with oilseed cake exhibited higher crude protein (CP), ether extract (EE), and non-fiber carbohydrates (NFC) values compared to rice straw without a protein source. Generally, the addition of roasted *Moramba* bean cake (RMBC) and whole sunflower seed cake (WSSC) to rice straw enhanced its CP content, suggesting a potential for valorization of rice straw. RMBC and WSSC provided mean CP values of 13% and 9.7%, respectively, both exceeding the minimum crude protein requirement of 7% for ruminants (McDonald *et al.*, 2011). Incorporating protein sources alongside non-fibre carbohydrates (NFC) reduced the fiber content in the diet, thereby increasing digestibility.

NFCs have significant benefits for ruminants as they ferment rapidly in the rumen, releasing sugars, pectin, and starch that promote microbial growth (Van Soest, 1994; Sun *et al.*, 2020).

According to Fortaleza *et al.* (2009), as cited in Antunes *et al.* (2019), oilseed cakes tend to increase the population of rumen microbes, which can result in reduced neutral detergent fiber (NDF) degradability. While feed with a high NDF content reduces rumen degradation (Soltan *et al.*, 2012), it improves the energy supply for ruminants.

4.5.2 *In vitro* pH changes

Maintaining rumen pH is crucial for the persistence and stability of intestinal microbes (Franzolin *et al.*, 2010). A stable rumen pH is necessary for optimal microbial activity, particularly for cellulolytic bacteria that break down fibrous components in the diet (Grant and Mertens, 1992). Although some selection of certain organisms undoubtedly occurred during *in vitro* fermentation, previous work (Dehority, 2003) has shown that energy-limited cultures produce a relatively normal fermentation pattern.

In the current study, the addition of oilseed cake to the rice straw (basal diet) did not alter the normal pH of rumen function and required minimal microbial adaptation. Despite the strong buffering capacity of the medium used, which apparently prevented a significant pH decrease, there was a slight reduction in pH for both nutrient inclusions of roasted Morama bean cake (RMBC) and whole sunflower seed cake (WSSC), observed at pH 6.43 and 6.55, respectively. These results indicate that different protein-enriched feed sources have varying effects on rumen pH changes.

Keunen *et al.* (2002) studied the effect of pH on various rumen microorganisms in continuous culture and observed concomitant decreases in pH and yield, partly due to a change in

fermentation products. High protein feeds are therefore able to neutralize acids and maintain a constant pH in continuous cultures (Dewhurst *et al.*, 2001). The rumen pH resulting from the diets observed in the present study promotes cellulolytic bacterial activity and fiber nutrition.

The extent of rumen fermentation varies between current protein diets and is influenced by interactions between diet composition and rumen microbial activity (Allen, 1997). The high in vitro dry matter disappearance (IVDMD) values observed for roasted Morama bean cake suggest that these feedstuffs have the potential to enhance nutrient utilization and support optimal growth and production in ruminants.

Overall, the combination of oilseed cakes with rice straw in the diets observed in the present study indicates the potential for these feedstuffs to enhance nutrient utilization and maintain an optimal rumen pH, supporting microbial activity and promoting the health and productivity of ruminants.

4.5.3 *In vitro* ammonia (NH₃) production

Rice straw at different concentrations mixed with oilseed cake showed consistent changes in ammonia concentration. The increase in ammonia levels in vitro suggested that rumen microbes (cellulolytic bacteria) utilizing it as a protein source could break it down and achieve optimal rumen fermentation rates of the feed. Therefore, an increase in ruminal NH₃-N concentration in rice straw mixed with oilseed cakes was a direct response to the provision of readily degradable nitrogen by the rumen microbes (Lintzenich *et al.*, 1995). This observation supports the findings of Van Soest (1994) and Broderick (1995), who reported that legume proteins are rapidly degraded in the rumen due to the accumulation of NH₃-N.

Moreover, the differences in ammonia production observed in the present study indicate variations in the solubility of crude protein content and CP degradability of the different feeds due to enzyme action (Uddin *et al.*, 2015; Mutsvangwa *et al.*, 2016). During feed consumption by ruminants, several rumen events can occur. Degradable crude protein in the rumen is broken down by the peptidases (secreted enzymes) of the proteolytic bacteria, converting peptides into amino acids (Putri *et al.*, 2021). The amino acids are then deaminated to release ammonia, which is used by the rumen microbes for protein synthesis (Ningrate *et al.*, 2019). When rumen degradable protein (RDP) in the diet exceeds the amount required by rumen microorganisms, the protein is broken down to $\text{NH}_3\text{-N}$, absorbed, metabolized to urea in the liver, and lost in urine (Bach *et al.*, 2005).

Despite the different crude protein concentrations of the feeds in the present study, ammonia production for both diets was above the critical value suggested by Leng (1990) (5 mg/100 ml), with the exception of rice straw without supplements (4.5 mg/100 ml). This helps meet the nitrogen needs of cellulolytic bacteria. The critical level of ammonia concentration refers to the level below 5 mg/100 ml that would be harmful or affect bacterial activity (Ørskov and Ryle, 1990). Previous investigators suggested critical ammonia concentrations of 5-7 mg/100 ml (Satter and Slyter, 1974), 15-20 mg/100 ml (Krebs and Leng, 1984), 4.5-12 mg/100 ml (Boniface *et al.*, 1986), and 26.4-30.9 mg/100 ml (Soltan *et al.*, 2012), which were requirements for minimal microbial activities in the rumen.

In the current study, ammonia concentrations from rice straw without supplements and from rice straw with oilseed cakes were between 4.5 and 15.3 mg/100 ml. These ammonia concentrations were within the ranges reported by previous workers (Boniface *et al.*, 1986; Soltan *et al.*, 2012;). The utilization of ammonia concentration by rumen bacteria depends on the availability of feed energy, rumen degradable protein, and a pH favourable for cellulolytic

bacteria (Dentinho *et al.*, 2014). Therefore, an increase in ammonia production in vitro leads to a decrease in volatile fatty acids (VFAs) in the rumen, and the proportion of urea in the total amount of nitrogen also decreases or vice versa (Soltan *et al.*, 2012).

The combined addition of oilseed cakes to rice straw in the diets observed in this study demonstrates the potential to enhance nutrient utilization and maintain an optimal rumen environment. The observed ammonia concentrations and their implications for microbial activity highlight the importance of considering the solubility and degradability of crude protein in feed formulations to support efficient rumen fermentation and overall animal health and productivity.

4.5.4 Volatile fatty acid production

The proportional fluctuations in volatile fatty acids (VFAs) resulting from feed fermentations were primarily due to the energy contribution of the different amounts of oilseed press cake (McDonald *et al.*, 2011). Rice straw without supplements had the lowest total volatile fatty acid content (8.22%) due to its low content of water-soluble carbohydrates (Wulandari *et al.*, 2020). Rumen VFA concentration is an important indicator of rumen microbial activity, and several studies have examined the effect of dietary crude protein (CP) levels on rumen fermentation, yielding inconsistent results. The present study suggests that CP levels increased linearly with an increase in dietary CP, leading to a corresponding increase in VFAs. These results are consistent with the findings of Pilachai *et al.* (2012), who reported that high levels of rumen-degradable protein (RDP) increased the concentrations of VFAs in the rumen, with higher concentrations observed in animals fed diets with increased RDP content. When ruminants consume feed high in CP, the protein is broken down by rumen microbes into amino acids and ammonia, providing the nitrogen necessary for microbial growth and proliferation.

These microbes then ferment carbohydrates, producing VFAs such as acetate, propionate, and butyrate, which are essential energy sources for the animal.

VFA production, including acetate, propionate, and butyrate, was significantly influenced by the supplementation of roasted *Morama* bean cake (RMBC) and whole sunflower seedcake (WSSC). RMBC supplementation resulted in higher VFA production compared to WSSC, particularly at higher inclusion rates, suggesting that RMBC is more effective in promoting fermentation and energy production in the rumen. The higher acetate production with RMBC supplementation indicates enhanced fermentation of structural carbohydrates, while the increased propionate production suggests improved energy availability for the animal.

Acetate production by different diets was affected by pH values above 6.0. Propionate production was lower when the pH of rice straw with supplements was 6.93, probably due to the nature of hexose, which had little effect on microbial function. Propionate is an energy-dense volatile fatty acid (VFA) used by ruminants as a precursor for gluconeogenesis (Mayland *et al.*, 1998). Butyrate production occurred at low concentrations in all feeds at different protein inclusion rates and was independent of pH. Franzolin *et al.* (2010) reported that microbial synthesis decreases when feed pH is 6.0 or below. The current observation indicates successful microbial protein synthesis, as the pH of *in vitro* degradation remained above 6.0 throughout the study.

In addition, total volatile fatty acids (TVFAs) were predicted based on *in vitro* dry matter degradability, true dry matter degradability, and organic matter degradability ($y = 0.4641x$, $R^2 = 0.9346$; $y = 0.3592x$, $R^2 = 0.97716$; $y = 0.4864x$, $R^2 = 0.9595$). The current results indicate that *in vitro* organic matter degradability can be used as an indicator for estimating volatile fatty acids in rice straw, with or without the addition of oilseed press cake. The breakdown of

organic matter is crucial in the diet of ruminants, as it includes protein fractions, fat, vitamins, and carbohydrates (Hafid and Patriani, 2020). Carbon from organic matter determines the structural backbone of pyruvate and acetyl coenzyme A, which play fundamental roles in biochemical reactions involving protein, carbohydrate, and lipid metabolism, thus contributing to VFA formation (Rahman *et al.*, 2013). The total amount of volatile fatty acids results from bacterial fermentation processes in the rumen and is ultimately converted into the bloodstream through a network of metabolic pathways to produce carbon dioxide, water, and energy in the form of ATP (Houston *et al.*, 1986).

Higher neutral detergent fiber (NDF) content, as observed in the rice straw and whole sunflower seed cake mix, indicated higher levels of structural carbohydrates. Higher structural carbohydrates, in turn, promote acetate production (Rahman *et al.*, 2013). Increased molar proportions of acetate in the rumen due to this mixture were associated with increased hemicellulose content of the NDF fraction, which would promote acetate-producing microbial species. In this context, the acetate-propionate ratio served as an indicator of the metabolic pathway used by rumen bacteria in the conversion of monosaccharides to volatile fatty acids (VFAs) (Stefanon *et al.*, 1996). Therefore, the lower acetate-propionate ratio observed in rice straw supplemented with sunflower seed cake suggested the presence of amylolytic bacteria in the rumen. In this case, the starch would be fermented to lactate by rumen bacteria that utilize the acrylate pathway to produce propionate (Van Soest, 1994).

The molar production of butyrate was observed to be lower for both protein feeds. Butyrate, a volatile fatty acid (VFA), plays an important role in energy metabolism and regulates genomic activities that influence nutrient utilization and function in the rumen (Gaebe *et al.*, 1998). However, the inclusion of a protein source improved the quality and degradability of rice straw. This observation is consistent with Tsopito (1999), who reported an increase in rumen butyrate

due to the gradual addition of soybean meal (SBM). Since the present study specified the addition of oil bean cake with no other additions, this could explain the lower butyrate values observed for both feed supplements.

4.5.5 Nutrient degradability

The dry matter (DM) and organic matter (OM) degradability of rice straw supplemented with oilseed press cake (RMBC and WSSC) increased due to the feed composition (Hanafi *et al.*, 2012). This was attributed to strong associative effects between the oilseed cakes and rice straw. Associative effects of feed occur when the use of one feed is dependent on the other, or when individual feed components are mixed to produce complementary proportions (Fattah *et al.*, 2020).

However, the inclusion of 30% RMBC resulted in a slight reduction in the in vitro degradability of DM and OM (Table 4.3). The lower microbial activity was influenced by the higher fat content (>5%) in the diet, which was toxic to rumen microbes and impaired fiber breakdown. This hypothesis is consistent with the findings of Makmur *et al.* (2020a), who reported lower feed intake in ruminants due to reduced microbial activity caused by increased fat supplementation.

In contrast, the diet supplemented with 30% roasted *Morama* bean cake (RMBC) showed a positive effect, demonstrated by a significant increase in bypass crude protein (BP-CP) and its digestibility in the hindgut. For rice straw without additives, the high amounts of silica and lignin likely impaired the activity of microbial enzymes that degrade cell wall carbohydrates (Hafid and Patriani, 2020). This hypothesis is supported by the findings of Elsayed and El-Samni (2006), who reported that 80% of rice straw is composed of silica, which often prevents microbial colonization (Bae *et al.*, 1997). Supplementing with RMBC offered the benefit of

supplying readily fermentable cellulose and hemicellulose (Table 4.1). Metzler-Zebeli *et al.* (2013) reported that fermentable cellulose and hemicellulose increase cellulolytic microbes, thereby improving the degradability of otherwise less degradable fiber.

In the supplemented groups (Figures 4.11, 4.13, and 4.14), orthogonal contrast analysis showed that the mean values of ivACPdeg, BP-CPdig, and ivACPdig of rice straw supplemented with RMBC were higher than those supplemented with WSSC (59.7 vs. 58.8; 31 vs. 28.3; and 16.2 vs. 14.1%, respectively). The inclusion of 20% RMBC resulted in a slightly lower mean BP-CP (Table 4.5). The increased BP-CP due to the inclusion of 30% RMBC is believed to be helpful in increasing protein flow to the duodenum (Soltan *et al.*, 2013).

The post-rumen dry matter digestibility (ivADMdig) of rice straw supplemented with RMBC was higher than all other feed treatments (Figure 4.10). The simulated digestion in the post-rumen was infused with hydrochloric acid at a low pH of 2, similar to that in a ruminant (Ansar *et al.*, 2014), which likely contributed to efficient digestion. After digestion, the amino acids are absorbed in the small intestine.

The study demonstrated that the addition of RMBC and WSSC to rice straw enhances its DM and OM degradability due to associative effects. While higher fat content in RMBC diets may reduce degradability, the inclusion of RMBC significantly increases bypass crude protein and post-rumen digestibility. The high silica and lignin content in rice straw without additives impairs microbial enzyme activity, but supplementation with RMBC improves the availability of fermentable cellulose and hemicellulose, enhancing fiber degradability and overall nutrient utilization.

4.6 Conclusion

The orthogonal contrasts analysis revealed significant linear and quadratic effects of the inclusion rates of roasted *Morama* bean cake (RMBC) and whole sunflower seed cake (WSSC) on dry matter (DM) and organic matter (OM) degradability, volatile fatty acid (VFA) production, and microbial activity in the rumen. Roasted *Morama* bean cake (RMBC) consistently outperformed whole sunflower seed cake (WSSC) in all measured parameters, including crude protein (CP), DM and OM degradability, VFA production, and microbial activity. The superior performance of RMBC is attributed to its higher nutritional quality and better digestibility compared to WSSC.

These findings indicate that supplementing feed with roasted *Morama* bean cake (RMBC) can significantly improve the degradability and fermentation efficiency of the feed, leading to better nutrient utilization and enhanced animal performance. However, it is important to determine the potential toxicology of this unconventional oil bean cake on rumen microbes to avoid the lysis of rumen microbes, which can cause metabolic disorders in ruminants when fed diets containing roasted *Morama* bean cake.

Furthermore, the *in vitro* technique can be effectively used to screen newly available agro-industrial by-products for protein degradability. However, for reliable estimates of protein degradability and the microbial efficiency of protein supplements, *in vivo* measurements (using a cannula in the duodenum) are preferred.

Overall, the findings highlight the potential of using *Morama* bean cake as an alternative protein source in ruminant diets, contributing to sustainable livestock production. The comparison demonstrates that RMBC is a superior feed supplement compared to WSSC, with

better outcomes in terms of nutrient utilization, microbial activity, and overall feed efficiency.

This supports the potential integration of RMBC into ruminant nutrition programs to enhance animal performance and promote sustainable livestock production.

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Chapter 5

5.1 General Discussion

In recent studies, the evaluation of *Morama* bean cakes and sunflower seedcake as dietary supplements for ruminants has yielded valuable insights into their nutritional benefits and impacts on rumen fermentation. The studies assessed roasted *Morama* bean cake (RMBC), whole *Morama* bean cake (WMBC), decorticated *Morama* bean cake (MBCD), and whole sunflower seedcake (WSSC) across various parameters, including dry matter (DM), crude protein (CP) and organic matter (OM) degradability, volatile fatty acid (VFA) production, and/or microbial activity.

5.1.1 Nutritional Composition

The chemical composition of *Morama* bean cakes and sunflower seedcake was a critical focus in all three studies. RMBC and MBCD were found to have higher crude protein (CP) and ether extract (EE) contents compared to WSSC. This superior nutritional profile of *Morama* bean cakes makes them excellent dietary supplements for providing both protein and energy to ruminants. The high CP content is particularly beneficial for supporting microbial growth and enhancing rumen fermentation.

5.1.2 Dry Matter (DM), Crude Protein (CP) and Organic Matter (OM) Degradability

All three studies consistently demonstrated that RMBC outperformed WSSC in terms of DM, CP, and OM degradability. The superior degradability of RMBC can be attributed to its better digestibility and higher nutritional quality. WMBC and MBCD also showed improvements in degradability compared to WSSC, although RMBC was the most effective. These findings

suggest that *Morama* bean cakes, especially RMBC, can significantly enhance the degradability of rice straw, leading to improved nutrient utilization.

5.1.3 Volatile Fatty Acid (VFA) Production

The production of VFAs, including acetate, propionate, and butyrate, was significantly influenced by the supplementation of *Morama* bean cakes and WSSC. RMBC consistently resulted in higher VFA production compared to WSSC, particularly at higher inclusion rates. This indicates that RMBC is more effective in promoting fermentation and energy production in the rumen. The higher acetate production with RMBC supplementation highlights its role in enhancing the fermentation of structural carbohydrates, while the increased propionate production suggests improved energy availability for the animal.

5.1.4 Microbial Activity

Microbial activity, as measured by ammonia (NH₃-N) concentration was higher with RMBC supplementation compared to WSSC. This finding indicates that RMBC provides a more favourable environment for rumen microbes, leading to enhanced protein degradation and utilization. The superior performance of RMBC can be attributed to its higher degradability and nutritional quality, which supports the growth and proliferation of rumen microbes. WMBC and MBCD also showed positive effects on microbial activity, further highlighting the benefits of *Morama* bean cakes.

5.1.5 Practical Implications

The results of these studies have important implications for ruminant nutrition and feed formulation. Supplementing rice straw with *Morama* bean cakes, particularly RMBC, can significantly improve the degradability and fermentation efficiency of the feed, leading to

better nutrient utilization and enhanced animal performance. Additionally, the findings highlight the potential of using *Morama* bean cakes as alternative protein sources in ruminant diets, contributing to sustainable livestock production.

5.2 General Conclusion

In conclusion, the consistent positive effects of *Morama* bean cakes across all measured parameters in the studies indicate their superior nutritional quality and effectiveness compared to sunflower seedcake. These findings support the incorporation of *Morama* bean cakes into ruminant diets to optimize feed efficiency and animal performance.

Using *Morama* bean cake (*Tylosema esculentum*) provides ruminants with cheap and valuable feed ingredients, which could reduce dependence on sunflower seed cake, which has become too expensive for small farmers. Roasted *Morama* bean cake is characterized by high nutritional value as it has a high crude protein content and a high lipid content, making it a suitable source of protein and energy for ruminants. The nutritional profile of animal feed is a crucial aspect of ruminant production as it directly impacts the health and productivity of the herd. The protocols used to determine chemical composition and nutrient utilization were derived from wet chemistry, *in vitro*, and *in situ* degradation.

Based on the results obtained, the following conclusions were drawn:

1. Different *Morama* bean cakes have different CP values depending on whether they were shelled, dehulled, and/or dehulled and roasted. The shelled or undecorticated *Morama* bean cake was comparable to sunflower seed cake in terms of CP content, while the rest of the *Morama* bean cakes had higher CP content.
2. The dehulled and roasted *Morama* bean cake (RMBC) had a significant influence on the effective DM and CP degradability. CP degradability slowed in *in situ* tests due to roasting,

which also increased protein content. This is a beneficial property of the protected protein for ruminants fed on nutrient-poor pastures, as it releases a lot of energy and supplies the small intestine with useful amino acids.

3. It is concluded that roasting *Morama* bean cake is an effective method for improving the rumen degradation of DM and CP by increasing the rumen-non-degradable protein fractions in the product. Therefore, roasting *Morama* bean cake could be useful to increase the proportion of rumen-non-degradable protein in the small intestine, provided that care is taken not to compromise the availability of amino acids in the small intestine.
4. Adding 20% roasted *Morama* bean cake to rice straw is most recommended as it has a higher content of bypass protein (BP-CP). Due to its lower CP degradability, it could be used in milk rations. On the other hand, rice straw could be used as a better fibre source for efficient microbial function in ruminants due to its higher NDF degradability.

In summary, supplementing ruminant diets with *Morama* bean cakes, particularly roasted *Morama* bean cake, offers substantial benefits in terms of improved degradability, nutrient utilization, and animal performance. The potential of *Morama* bean cakes as alternative protein sources contributes to sustainable livestock production and supports small farmers by providing an affordable and effective feed option.

5.3 Recommendations

In light of the conclusions drawn from this study, the following recommendations are proposed:

Mineral Content of *Morama* Bean Cakes: *Morama* bean cakes are a valuable source of essential minerals, including calcium, potassium, magnesium, sodium, iron, selenium, copper, and zinc. It is recommended to further explore the mineral profile of *Morama* bean cakes to better understand their potential in meeting the mineral requirements of ruminants.

In Vivo Experiments: This study was conducted without the use of animal feeding studies to demonstrate the degradability, digestibility, nitrogen retention, growth, and health effects of feeding *Morama* bean cake. Therefore, it is recommended to conduct *in vivo* experiments to validate the findings and assess the practical implications of feeding *Morama* bean cake to ruminants.

Optimal Crude Protein (CP) Levels: A medium CP value in the diet may be more effective in preventing nitrogen waste compared to lower or higher CP levels. It is suggested that a CP level of 13-14% is more appropriate than the 19% observed at 300g/kg RMBC in the diet when the concentrate ration is provided at a high level. Further research is recommended to determine the optimal CP level for different feeding scenarios.

Inclusion of Yeast or Urea: It is recommended to evaluate the potential benefits of including yeast or urea in the basal diet of low-quality roughage, in addition to *Morama* bean cakes. These supplements could improve digestibility and overall nutritional value for ruminants.

Combination with Other Energy Supplements: Supplementation with *Tylosema esculentum* (*Morama*) cakes in combination with maize kernels or maize kernels and sorghum bran is advised. Using multiple energy supplements can achieve better protein-energy synchronization and improve nutrient utilization.

N-Polluting Effect: The possibility of nitrogen pollution from animal feed supplementation with *Morama* bean cake should be examined in detail, particularly as part of metabolism studies. This will help in understanding the environmental impact and developing sustainable feeding practices.

Methane Emissions: The lipid/fat content in *Morama* bean cakes may have the potential to reduce methane emissions from ruminants. Further research is needed to quantify this effect and explore its potential for mitigating greenhouse gas emissions.

Calcium to Phosphorus Ratio: The study indicated that *Morama* bean cakes had a low calcium to phosphorus ratio. Future research should investigate how this might affect grazing animals and what corrective measures can be taken by supplementing the feed with the right balance of required minerals.

Research on Different Animal Categories: Additional research on various animal categories at different growth stages is needed to gain detailed insights into the effects of *Morama* bean cake supplementation. This will help determine the acceptable dietary supplementation levels without adverse effects on ruminants.

By addressing these recommendations, further research and practical applications can enhance the utilization of *Morama* bean cakes in ruminant diets, contributing to improved animal performance and sustainable livestock production.