Techniques used to increase the rumen undegradable protein fraction in locally produced plant protein sources

by

Olga Dreyer

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Supervisor: Prof Tertius Swanepoel Brand

Co-supervisor: Dr Johan Hendrik Combrink van Zyl

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Declaration

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Summary

Title	:	Techniques used to increase the rumen undegradable protein fraction in locally produced plant protein sources
Candidate	:	Olga Dreyer
Supervisor	:	Prof T.S. Brand
Institution	:	Department of Animal Sciences, Stellenbosch University
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With pressure directed towards South African farmers to fulfill the increasing mutton and lamb demand along with increasing commodity prices, the necessity presents itself to make use of more affordable, readily-available, locally produced plant protein sources to satisfy the animals' nutritional demands. Due to protein being an important nutritional aspect of a ruminant's ration it is of utmost importance to ensure optimal utilization of the protein source. Processing methods such as extrusion and formaldehyde treatment increase the amount of rumen undegradable protein (RUP), enabling the animal to achieve its optimal genetic potential. This is due to the possible increased protein available for absorption in the small intestines. With the Southern Cape and Swartland regions being large producers of lupin and canola, these high-quality plant proteins could potentially be used as replacement protein sources that are more affordable, relative to fishmeal and imported soybean meal. The need for lamb intensification systems and feedlot finishing also presents itself, in order for South Africa's lamb and mutton operations to supply the growing population's demand. Thus, both strategies of sheep production intensification and the utilization of protected, more affordable plant protein sources could potentially act as a possibility to increase the small ruminant sector's annual revenue and optimize the farmers' operational sustainability and profitability.

The aim of the study was to determine the effect of extrusion (Trial 1) and formaldehyde treatment (Trial 2) on the rumen undegradable protein fraction of locally produced plant protein sources, canola oilcake meal (CM) and sweet lupins (SL).

The effect of extrusion (Trial 1) was determined using 56 Meatmaster lambs (live weight *ca* 22.9 kg). Lambs were housed at the metabolism facilities at Elsenburg, where they were placed in individual paddocks each supplied with *ad libitum* feed and water. Each lamb was supplied with a specific concentrated feed, based on the treatment group allocated to them. The four treatment groups were canola oilcake meal control (CC), canola oilcake meal extruded (CE), sweet lupin control (LC) and sweet lupin extruded (LE). Lambs were randomly allocated to the respective groups, with each group

consisting of 16 lambs (7 ewes and 9 rams per group). No significant interaction was present for protein source and extrusion processing. A feed conversion ratio of 4.62 *versus* 4.85 kg feed/kg weight gain was present in the study for the extruded diet *versus* the control diet. An average daily gain of 0.310 kg/day and 0.320 kg/day were obtained for the control diet relative to the extruded diet. Similarly, no effect of extrusion on daily feed intake was observed between CM and SL. The production performance of lambs that received either CM (17.4% inclusion level) or SL (26.6% inclusion level) did not differ significantly. An average fat score of 4 was achieved, with no effect of processing or protein source on the carcass fat thickness. However, SL were identified as an inexpensive locally produced plant protein source, in comparison to canola oilcake meal, which obtains similar performance parameters in lambs under feedlot conditions in this study.

The effect of formaldehyde treatment (Trial 2) was determined by using six Dohne Merino wethers (live weight of *ca* 95 kg), already fitted with rumen cannulas, housed at Kromme Rhee Experimental Farm, in order to determine the *in situ* crude protein (CP) and dry matter (DM) degradability. Each animal was placed in its individual paddock. They were fed an *ad libitum* basal diet consisting of 50:50 wheat straw and lucerne hay. Canola oilcake meal (CM) and sweet lupin seed (SL) were treated with formaldehyde (40% w/v) at concentrations of 10g (F10) and 15g/kg CP (F15). The treatments entailed CM control (CMF0), CM treated with 10 g/kg CP formaldehyde (CMF10), CM treated with 15 g/kg CP formaldehyde (CMF15), SL control (SLF0), SL treated with 10 g/kg CP formaldehyde (SLF10) and SL treated with 15 g/kg CP formaldehyde (SLF15). Treatments were incubated in the rumen at time intervals of 0, 2, 4, 12, 36, 48, 72, and 96 hours.
Formaldehyde significantly decreased the soluble fraction and lowered the rate of degradation of the potential degradable fraction at both concentrations. The DM effective degradation was significantly decreased at all outflow rates with the largest effect seen at 0.08/h. Both treatment concentrations decreased DM effective degradation by 16.1% and 20%, respectively. Formaldehyde treatment also significantly decreased the CP effective degradation (27.9% at F10 and 31.1% at F15) at all outflow rates, with the largest effect seen at 0.08/h. Overall, formaldehyde treatment effectively decreased DM and CP rumen degradation at all outflow rates of both CM and SL. Therefore, formaldehyde treatment could be used to increase the rumen undegradable protein fraction. Potential improvement in animal performance in terms of live weight gain, average daily gain, and feed conversion efficiency has to be done in production studies.

Opsomming

Titel	:	Metodes wat gebruik kan word om die rumen onafbreekbare proteïen fraksie in plaaslik geproduseerde plant produkte te verhoog
Kandidaat	:	Olga Dreyer
Studieleier	:	Prof T.S. Brand
Instelling	:	Departement Veekunde, Stellenbosch Universiteit
Graad	:	MSc Agric (Veekunde)

Met druk wat op Suid-Afrikaanse boere gerig word om aan die toenemende skaap- en lamsvleis aanvraag te voldoen, tesame met die stygende kommoditeitspryse, ontstaan die noodsaaklikheid om van meer bekostigbare, relatief beskikbare, plaaslik geproduseerde plant proteïene gebruik te maak.

Omrede proteïene ‘n belangrike voedingsaspek van ‘n herkouer se rantsoen is, is dit van uiterste belang om optimale benutting van die proteïenbron te verseker.

Prossesseringsmetodes soos ekstrusie en formaldehiedbehandeling, verhoog die hoeveelheid rumen onafbreekbare proteïene (RUP) wat die dier in staat stel om sy optimale genetiese potensiaal te bereik, as gevolg van verhoogde proteïen beskikbaarheid in die dunderm.

Met die Suid Kaap en Swartland-streke as groot produsente van lupiene en kanola, kan hierdie hoë kwaliteit plant proteïene moontlik gebruik word as alternatiewe proteïen bronne wat meer bekostigbaar is relatief tot vismeel en ingevoerde sojaboon meel.

Die behoefte aan intensiewe lamstelsels en voerkraal afronding doen hom voor, sodat Suid Afrika se lam en skaapvleis industrie in die groeiende bevolking se aanvraag voldoen. Dus kan beide strategieë van skaap produksie intensifisering en die gebruik van meer bekostigbare plant proteïen bronne moontlik dien as strategie om die herkouers sektor se jaarlikse inkomste te verhoog en die produsent se bedryfsvolhoubaarheid en winsgewendheid te optimaliseer.

Die objektief/mikpunt van die studie was om die effek van beide ekstrusie en formaldehiedbehandeling te bepaal, om die RUP te verhoog van plaaslik, geproduseerde plant proteien bronne, kanola oliekoek meel (CM) en soet lupiene (SL).

Die effek van ekstrusie (Proef 1) is met behulp van Meatmaster lammers (lewenede massa ± 22.9kg) bepaal. Lammers is gehuisves by Elsenburg se metabolisme fasiliteite waar hulle in individuele hokke geplaas is, waar elkeen *ad libitum* voer en water voorsien is.

Die vier behandelingsgroepe was kanola oliekoek kontrole (CC), kanola oliekoek geekstrueer (CE), soet lupien kontrole (LC) en soet lupien geekstrueer (LE). Lammers is ewekansig verdeel in behandelingsgroepe, met elke groep bestaande uit 16 lammers (7 ooie en 9 ramme).
Geen betekenisvole interaksie was teenwoordig vir proteïenbron of ekstrusie prosessering nie. ‘n Voeromsetverhouding van 4.62 in vergelyking met 4.85kg voer/kg gewigstoename was teenwoording in die studie vir die geekstrueerde voer teenoor die kontrole voer. Netso, is geen effek van ekstrusie op die daaglikse voerinname tussen CM en SL waargeneem nie. Produksie van lammers wat CM (17.4% insluitingsvlak) of SL (26.6% insluitingsvlak) ontvang het, het nie betekenisvol verskil nie. ‘n Gemiddelde karkas vetgradering van 4 is behaal, met geen effek van ekstrusie prosessering of proteïenbron op die karkas vetdikte nie.
Soet lupiene is egter geidentifiseer as ‘n goedkoper, plaaslik geproduseerde plant proteïen bron, in vergelyking met kanola oliekoek meel, waar Meatmaster lammers soortgelyke produksie data onder voerkraal toestande verwerf het.

Die effek van formaldehied behandeling (Proef 2) is *in situ* bepaal deur gebruik te maak van 6 Dohne Merino skape (lewende massa ± 95kg), reeds toegerus met rumen fistulas, gehuisves by Kromme Rhee Proef plaas om die degradering van ru-proteïene en droëmaterial in die rumen te bepaal. Individuele hokke per skaap is gebruik gedurende die proef. ‘n *Ad libitum* dieët bestaande uit 50:50 koringstrooi en lusernhooi is gevoer. Kanola oliekoek meel (CM) en soet lupien saad (SL) is behandel met formaldehied (40% w/v) by konsentrasies van 10g/kg (F10) en 15g/kg CP (F15). Die behandelings bestaan uit kanola oliekoek meel kontrole (CMF0), kanola oliekoek meel behandel met 10g/kg CP formaldehied (CMF10), kanola oliekoek meel behandel met 15g/kg CP formaldehied (CMF15), soet lupien kontrole (SLF0), soet lupien behandel met 10g/kg CP formaldehied (SLF10) en soet lupien behandel met 15g/kg CP formaldehied (SLF15).
Die behandelings was by 0 (kontrole), 2, 4, 12, 36, 48, 72 en 96 uur in die rumen geïnkubeer.
Formaldehied behandeling het die oplosbare fraksie en die tempo van degradasie van die potensiële afbreekbare fraksie, by beide behandelings konsentrasies verlaag. Die droëmateriaal (DM) is aansienlik verminder by alle uitvloei tempos met die grootste effek by 0.08/h. Beide behandelings konsentrasies het die DM effektiewe degradasie drasties verminder by alle uitvloei tempos, met die grootste effek teen 0.08/h (F10 verminder met 16.1% en F15 vermider met 20%). Formaldehied behandeling het ook die CP effektiewe degradasie by alle uitvloei tempos drasties verminder, met die grootste effek by 0.08/h (F10 verminder met 27.9% en F15 verminder met 31.1%).
Dus kan formaldehied behandeling gebruik word om die rumen onafbreekbare proteïen fraksie (RUP) te verhoog, wat lei tot die potensiële verbetering in diere prestasie in terme van lewende gewigstoename, gemiddelde daaglikse toename en voeromset doeltreffendheid.

This thesis is dedicated to

my parents (Mario & Cecile Dreyer).

Thank you for all your support and encouragement to follow my passion

Biographical sketch

Olga was born and grew up in Paarl, Western Cape, South Africa. Her love and passion for animals began in her early childhood when she was particularly fascinated with milk calves and horse riding and continuously wanted to go visit the farm. At the age of fifteen years, she started to job shadow and discovered animal science, particularly the feed industry. With immediate certainty, she knew that this was the career path for her. She matriculated at High School Paarl Gymnasium in 2016 and enrolled for BSc Agric Animal Science in 2017 at Stellenbosch University. During her undergraduate studies, she was passionate about extending her knowledge and therefore completed a variety of vacation work on livestock farms. With her main interest in ruminants, specifically beef and sheep nutrition, she applied for a post-graduate project. Inspired by Prof T.S. Brand's project and based upon excellent results obtained from a former study, she wholeheartedly pursued her MSc Agric studies, with the dissertation title "Techniques to improve the non-degradable protein fractions of locally produced plant protein sources".

It is a vision of hers to implement the knowledge and experience she has gained during her studies to assist farmers in accelerating and improving their production parameters. With unlimited potential in the agricultural sector, it is her goal to contribute positively to this industry with the ambition to keep on growing and working towards success.

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List of Abbreviation

- ***A*** Soluble protein fraction (%)
- **ADF** Acid detergent fibre
- **ADG** Average daily gain
- **ADIN** Acid detergent insoluble nitrogen
- **AFMA** Animal Feed Manufacturers Association
- ***B*** Fraction degraded over time (%)
- **BCFA** Branched-chain fatty acids
- ***C*** Rate of degradation of the *b* fraction (%/h)
- ***ca*** Approximately
- **Ca** Calcium
- **CC** Canola oilcake meal control (Chapter 3)
- **CE** Canola oilcake meal extruded (Chapter 3)
- **CF** Crude fibre
- **CMF0** Canola oilcake meal treated with no formaldehyde (0g/kg CP) (Chapter 4)
- **CMF10** Canola oilcake meal treated with 10g/kg CP formaldehyde (40% v/w) (Chapter 4)
- **CMF15** Canola oilcake meal treated with 15g/kg CP formaldehyde (40% v/w) (Chapter 4)
- **CO_2** Carbon dioxide
- **CP** Crude protein
- **DAEC** Departmental Evaluation Committee
- **DM** Dry matter
- **EU** European Union
- **FCE** Feed conversion efficiency
- **FCR** Feed conversion ratio
- **FI** Feed intake
- **HCHO** Formaldehyde
- **HTST** High-temperature short time
- **IVGPT** *In vitro* gas production technique
- ***k*** Fractional rumen outflow rate
- **LC** Sweet lupin control (Chapter 3)
- **LE** Sweet lupin extruded (Chapter 3)
- **MJ** Mega Joule
- **N** Nitrogen
- **NDF** Neutral detergent fibre

- **NPN** Non-protein nitrogen
- **OMD** Organic matter digestibility
- **P** Phosphate
- ***p*** potential degradation at the time, *t*
- **PRF** Protein Research Foundation
- **RDP** Rumen degradable protein
- **RUP** Rumen undegradable protein
- **SAA** Sulphur containing amino acid
- **SAS** Statistical Analysis Software
- **SI** Small intestines
- **SLF0** Sweet lupin seed treated with no formaldehyde (0g/kg CP) (Chapter 4)
- **SLF10** Sweet lupin seed treated with 15g/kg CP formaldehyde (40% v/w) (Chapter 4)
- **SLF15** Sweet lupin seed treated with 10g/kg CP formaldehyde (40% v/w) (Chapter 4)
- ***t*** time (h)
- **TDN** Total digestible nutrients
- **TP** True protein
- **UDP** Undegradable protein
- **UP** Undegraded protein
- **VFA** Volatile fatty acids

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Preface

This thesis is presented as a compilation of five chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Animal Science to which Chapter 3 was submitted for publication.

Chapter 1 General introduction

Chapter 2 Literature review

Chapter 3 The effect of extrusion of canola oilcake meal and sweet lupins on the production performance of Meatmaster lambs under feedlot conditions

Chapter 4 The effect of formaldehyde treatment of canola oilcake meal and sweet lupins on the *in situ* dry matter and crude protein digestibility

Chapter 5 General conclusions and future recommendations

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Chapter 1

General Introduction

Increasing lamb and mutton prices, animal feed prices, and rivalry between the food and feed sector are key components affecting profitability within a farming operation. The enduring rise in meat prices steers farmers in the direction of intensifying their sheep finishing systems to produce early market-ready lambs (Van Der Merwe *et al.*, 2020). The Department of Agriculture Land Reform and Rural Development (2021) indicated increases of farming requisites up to a 10% increase in animal feed, as a result of seed and fertilizer prices that increased by 10% and 4%, respectively. Additionally, the consumers' food prices increased by 5.7%, with meat prices increasing by 6.6%. Thus, the overall average price of animal products increased by 2.2% (Department of Agriculture Land Reform and Rural Development, 2021).

Due to increasing farming operational costs, the opportunity presents itself to use processing methods, such as extrusion (physical processing) and formaldehyde treatment (chemical processing) to potentially increase the rumen undegradable protein fraction in feedstuffs. Locally produced plant protein sources, such as canola oilcake meal (*ca* 21% CP on DM-basis) (Blair, 2011) and sweet lupins (*ca* 40% CP on DM-basis) (Nwokolo & Smartt, 1996) are more within financial reach, compared to protein sources such as fishmeal.

Lupins play an important role as an alternative crop produced in the Western Cape region of South Africa (Brand & Brandt, 2000), with canola ranked globally as the crop with the third highest production of oilseed (Brand *et al.*, 2001). With canola oilcake meal and sweet lupins that are both highly degradable in the rumen (77% and 81%, respectively) (Jordaan & Brand, 2020), (See comment in Literature list) extrusion and/or formaldehyde treatment could potentially optimize the animals' use of these plant protein sources. Regarding the inclusion of a protein source in fattening diets, factors such as protein source price (R/ton), availability and crude protein content of the feed must be considered (Brand *et al.*, 2001).

Extrusion entails a physical process that modifies the functional characteristics of feed by changing their structure and rendering the protein less susceptible to rumen degradation (Solanas *et al.*, 2008). Previous research indicated that extrusion decreases rumen protein degradability, increases the rumen undegradable protein fraction, enhances the nitrogen balance, and improves overall animal performance (Chalupa, 1975; Konishi *et al.*, 1999). However, extrusion temperature application is extremely important to prevent heat damage that could have an irreversible effect on the protein. Shortcomings may include the variation between different research studies, due to extrusion processing conditions not always being well defined (Serrano *et al.*, 1998). Moderate heat application in some studies also resulted in a lack of response (no definite effect on animal

performance) obtained from the feed being extruded. Nonetheless, extrusion is an environmentally friendly process that produces less waste products relative to other heating processes. ExtruAfrica stated that manufacturers could potentially save 19% on raw materials, 44% on capital investment, and 14% on labour by utilizing extrusion processing (Claassen, 2011).

Formaldehyde (HCHO) treatment of feedstuffs consists of chemical reactions between the protein and HCHO, where formaldehyde renders the feedstuff resistant to rumen degradation. A variety of previous research stated that formaldehyde treatment increases the rumen undegradable protein fraction (RUP), decreases rumen degradation, has a positive effect on intestinal digestibility of protein and increases animal production (Serrano *et al*., 1998; Van Straalen, 1995; Eghbali *et al.*, 2011). One aspect of formaldehyde treatment that raises objection is the carcinogenic factor. However, studies have proven that the application of HCHO in animal feed, does not affect the animal (Gulati *et al.*, 2005). Formaldehyde is not a carcinogen when consumed orally (Gulati *et al.*, 2005). Nonetheless, human safety measurements (such as gloves, masks, enclosed storage facilities, and eyewear) must be administered when HCHO treatment is applied. This is due to HCHO vapour causing sensitivity to human sensory systems.

The current study aims to identify if extrusion increases the RUP in locally produced plant protein (canola oilcake meal and sweet lupins); also to determine its overall effect on the production performance of lambs under feedlot conditions. Furthermore, in terms of HCHO treatment, the study aims to determine if chemical treatment decreases the effective crude protein (CP) and dry matter (DM) degradation in the rumen, ultimately increasing the RUP fraction of the feedstuffs (canola oilcake meal and sweet lupins).

Two separate research studies were performed. A feeding trial commenced, where lambs were kept under feedlot conditions and fed four different concentrate diets to determine the effect of extrusion and protein source. Furthermore, a second trial entailing an *in situ* study was performed on formaldehyde-treated samples.

The objectives of the research study were to determine:

- The effect of extrusion of canola oilcake meal on the production performance of young lambs kept under feedlot conditions.
- The effect of extrusion of sweet lupins on the production performance of young lambs kept under feedlot conditions.
- The effect of formaldehyde treatment of canola oilcake meal on the *in situ* degradability of protein.
- The effect of formaldehyde treatment of sweet lupins on the *in situ* degradability of protein.

In conclusion, this research study hypothesizes that the extrusion of both plant protein sources (canola oilcake meal and sweet lupins) will increase the production performance of young lambs under feedlot conditions, along with formaldehyde treatment decreasing rumen crude protein degradation and increasing the rumen undegradable protein fraction.

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Chapter 2

Literature Review

2.1 Introduction

With pressure directed toward South African farmers to fulfill the increasing mutton and lamb demand (Van Der Merwe *et al.*, 2020), along with increasing commodity prices, the necessity presents itself to make use of more affordable, readily-available, locally produced plant protein sources to satisfy the animals' nutritional demands.

Due to protein being an important nutritional aspect of a ruminant's ration (Garg, 1998), it is of utmost importance to ensure optimal utilization of the protein source. Processing methods such as extrusion and formaldehyde treatment increase the amount of rumen undegradable protein (RUP), enabling the animal to achieve its optimal genetic potential. This is due to the possible increased protein available for absorption in the small intestines. With the Southern Cape and Swartland regions being large producers of lupin and canola, these high-quality plant proteins could potentially be used as replacement protein sources that are more affordable, relative to fishmeal and imported soybean meal.

The need for lamb intensification systems and feedlot finishing also presents itself, in order for South Africa's lamb and mutton operations to supply the growing population's demand. Thus, both strategies of sheep production intensification and the utilization of protected, more affordable plant protein sources could potentially act as a possibility to increase the small ruminant sector's annual revenue and optimize the farmers' operational sustainability and profitability.

2.2 Protein metabolism in ruminants

Ruminants have the ability to digest poor-quality feed sources and convert it into high-quality end products, due to their four-compartment stomach (the rumen, reticulum, omasum, and abomasum). The rumen, making up 85 % of the volume (Van Der Honing, 1988),is the largest compartment consisting of a specialized microbial population, with *Bacteroides*, *Butyrivibrio*, and *Selenornollas* species being the dominant proteolytic rumen bacteria (Chalupa, 1974), which digests the feed consumed, and in turn, supplies the animal with the required metabolites used for production.

For a ruminant to achieve its optimal genetic potential, its feeding requirements in terms of protein, amino acids, energy, fat, starch, and fibre must be adhered to. With protein being one of the most crucial constituents in a ruminant's ration, it is essential to ensure that it is utilized with high efficiency (Garg, 1998). Figure 2.1 represents a ruminant animal's digestive system in terms of protein, urea, and carbohydrate utilization.

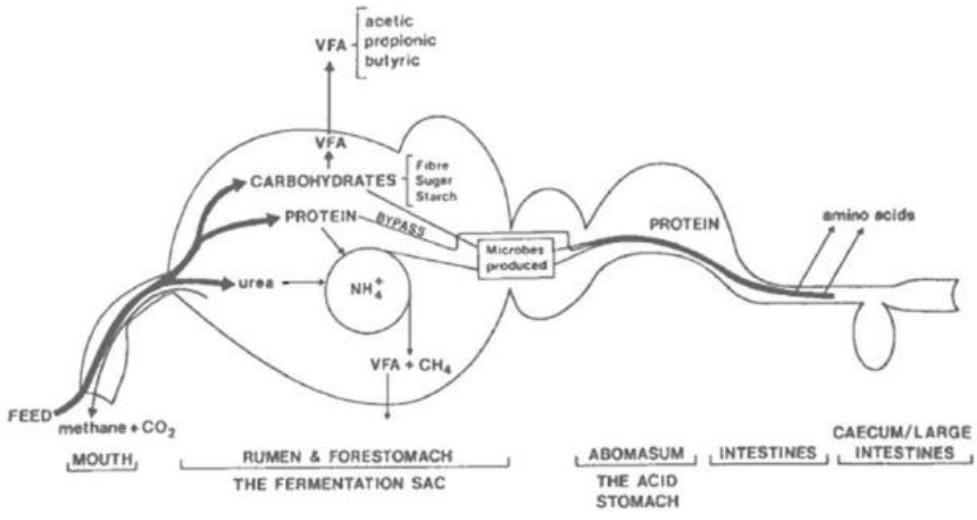

Figure 2.1 Schematic representation of a ruminant's digestive system showing the utilization of protein, urea and carbohydrates (Brand, 1996). Comment: Not in Reference list

True protein can be divided into rumen degradable protein (RDP) and rumen undegradable protein (RUP), also known as bypass protein (Brand & Jordaan, 2020b). The rumen degradable protein does not escape rumen degradation and is broken down by rumen microorganisms (Van Der Honing, 1988) into peptides and ammonia. Ammonia acts as the primary nitrogenous source which is utilized by the microbes for the production of microbial protein (McDonald, 1947). Ammonia is either directly absorbed via the rumen wall, passed out the rumen *via* the fluid digesta phase, or converted to microbial crude protein (Kempton & Leng, 1971). As much as 80% of the dietary protein degraded could potentially be transferred to microbial crude protein (Chalupa, 1974). Production of the microbial crude protein is dependent on the energy supplied from the degradation of carbohydrates in the feed. Thus, a considerable part of the protein being supplied through the diet should be fermentable in the rumen to meet the nitrogen requirements of micro-organisms to obtain microbial growth and fibre digestion (Garg, 1998).

However, the rumen undegradable protein or bypass protein escapes rumen degradation and is converted to amino acids for utilization in the small intestines and abomasum, with the abomasum being the main absorption site. The amino acids available for absorption are the result of those available from the microbial proteins and feed proteins which bypass rumen degradation and are further exposed to enzymatic digestion. Thus, the affiliation between microbial protein synthesis and energy supply *via* carbohydrate degradation is principal to the protein metabolism in ruminants (Garg, 1998). Therefore, the total dietary protein transformed to microbial protein acts as an indication of the nitrogen status of the ruminant and should be used as a determining factor whether to increase or decrease ruminal degradation by simulated procedures (Chalupa, 1975).

2.3 Protein requirements of ruminants

Although ruminants are effective users of a variety of protein feed sources, including low-quality protein by-products and crop residues in the agro-industry (Kempton & Leng, 1971), there is, however, a diversity of factors influencing their protein requirements. In past research studies, the dietary protein requirements of a ruminant animal were based upon the crude protein (nitrogen x 6.25) content. This, however, faded due to ruminants mostly obtaining their essential amino acids from the microbial protein in the rumen. Thus, the protein requirements are in terms of the amount of non-protein nitrogen or rumen degradable protein and amino acid-nitrogen required by the animal (Kempton & Leng, 1971).

2.3.1 Factors influencing protein requirements

The key factors determining protein requirements vary in terms of the animal's production stage and the physiological status of the animal, whether it being milk production, wool growth rate, or stage of pregnancy. Additional factors affecting protein requirements include the animal's glucose requirement, and ruminal fermentation, which directly influences the availability of volatile fatty acids such as isobutyric acid, valeric acid, and propionic acid (Leng, 1976). The efficiency of microbial protein synthesis (Thomas, 1973) should also be taken into consideration when determining the protein requirements (Kempton & Leng, 1971).

2.4 Protein degradation

2.4.1 Rumen degradation of protein

Dietary crude protein (CP) is defined as the nitrogen content of a feedstuff, multiplied by 6.25, which is the factor consequential to the average vegetable protein's nitrogen percentage (Visagie, 2010). The three main fractions into which CP can be subdivided, include true protein (TP), non-protein nitrogen (NPN), and acid-detergent insoluble nitrogen (ADIN). True protein is sectioned into RDP and RUP. As mentioned previously, anaerobic protein degradation is divided into an initial hydrolyzation step where amino acids and peptides are formed, followed by the deamination producing CO_2, VFA's, BCFA's, and ammonia (Van Straalen, 1995).

The exact protein requirements of a high-producing ruminant animal are of vital importance, due to the nutritional protein pertaining to an important role in the production of ruminal ammonia (Poos-Floyd *et al.*, 1985). Shannak *et al.* (2000) stated that ruminal protein degradation is prominently dependent on the amount of soluble and insoluble protein and the rate at which digestible and indigestible protein is degraded, with 65-75% apparent absorption of amino acid nitrogen occurring in the small intestines (SI) (Van Straalen, 1995).

2.4.2 Aspects influencing protein degradation

A broad spectrum of previous research articles identified various factors influencing protein degradation, with the main factors being protein solubility and the retention time in the rumen (Kempton & Leng, 1971; Chalupa, 1974). Additional factors are the ruminal pH, microbial proteolytic

rumen activity, and microbial access to protein (Visagie, 2010). Visagie (2010) established that the retention time is subject to the level of feed intake and the particle size of the diet components. Sheep rumen retention time is reported as being 0.8 to 2.2 days, with retention time varying between different diets, animals of the same species, and different species. The rate of digestion, rate of passage, the solubility of the protein in rumen fluid and quantity of protein ingested, independently influence the extent to which protein is degraded in the rumen (Visagie, 2010). Thus, the rate at which protein is degraded (Orskov & McDonald, 1979) consequentially affects the amount of UDP reaching the main absorption site, due to influencing microbial protein synthesis. This is a crucial factor enabling the microbes to supply microbial protein, which in turn provides amino acids passed on to the small intestines, being available to the animal for their production requirements. Nonetheless, the rate of rumen protein degradation could also be influenced by management strategies such as altering the level, ratio, and frequency of supplying the animals with either a roughage or concentrated diet (Van Straalen, 1995).

2.5 Techniques to evaluate protein quality and ruminal degradation

2.5.1 *In vivo* technique

The *in vivo* technique is effective in testing for performance in feeding or metabolism trials to examine the effectiveness of a certain product or feed known to enhance protein degradability (Jordaan & Brand, 2020). The method involves the utilization of the total flux of digesta through the digestive tract of the animal by making use of feed markers (Jordaan & Brand, 2020). These feed markers are essential in differentiating between the dietary nutrients and microbial protein, along with determining the flow rate of digesta occurring in the SI (Shannak *et al.*, 2000). However, the *in vivo* technique is not very practical due to having a slow turnover rate, being expensive and labour intensive, along with potential errors involving the feed markers used. The technique is also inconvenient in testing an individual feed source, due to the animal's metabolic requirements to consume a balanced diet (Jordaan & Brand, 2020).

2.5.2 *In vitro* technique

The *in vitro* method to determine the digestibility of a feedstuff is based on the solubility of protein as an index of digestibility (Krishnamoorthy *et al.*, 1983). With regard to the *in vitro* technique, feed samples are incubated in rumen fluid in an environment that simulates the rumen in terms of movement and heat, with degradability that could be measured at different time intervals (Jordaan & Brand, 2020). A previous research study identified the *in vitro* technique as being a rapid, cost-effective method that allows the observation of the dynamics of the breakdown of proteins by only requiring small amounts of raw material (Eid & Matty, 1989).

An additional method used specifically for feed evaluation is the *in vitro* gas production technique (IVGPT) (Das *et al.*, 2015). However, IVGPT is an alternative option, but it is criticized for utilizing a waste product from fermentation and not directly representing the rate of degradability (Jordaan & Brand, 2020).

2.5.3 *In situ* technique

The *in situ* technique is a mixture of the *in vitro* and *in vivo* methods (Jordaan & Brand, 2020). It involves the incubation of a nylon or dacron bag (pore size between 30 to 50 microns) (Van Straalen, 1995) in the rumen of a fistulated animal (Poos-Floyd *et al.*, 1985), to obtain a representative evaluation with regards to the feed's ruminal degradation (Ørskov *et al.*, 1980; Das *et al.*, 2015). Ørskov *et al*. (1980) developed a model by utilizing the *in situ* method to attain a predictable rate of digestibility in the rumen based on passage rate. This technique is renowned globally for determining rumen CP degradation, along with obtaining UDP values in a short period (Shannak *et al.*, 2000). The Cornell net carbohydrate and protein system (CNCPS) identified the *in situ* technique as being an accurate and dependable method in estimating the UDP values *in situ* and chemical fractions of feed protein (Shannak *et al.*, 2000).

The dracon/nylon bag technique represents the potential degradation (p) using the following equation $p = a + b\,(1 + e - ct)$, with a, b; c being constants and t the retention time in the rumen (Van Straalen, 1995). The equation produces a degradation curve used in estimating the ruminal degradation rate. The greatest benefit of this technique is that the dracon/nylon bag allows a better understanding of feedstuff degradation in the rumen (Ørskov *et al.*, 1980). Nevertheless, four shortcomings of this technique are (i) no exposure to breakdown (such as rumination and or chewing), (ii) the sample in the nylon bag is only degraded up to a size suitable to leave the bag, (iii) contamination of the sample with microbial protein (Van Straalen, 1995) and lastly (iv), no feedstuff escapes the rumen. It is estimated that 5g concentrate samples are suitable for rumen incubation with an approximate 12 to 36-hour incubation period (Ørskov *et al.*, 1980).

2.5.4 Enzyme assays

This method utilizes proteolytic enzymes. The enzymes represent maximum activity under ruminal environmental conditions, with pH 5-7 and a ruminal temperature of 35 to 45 ºC (Poos-Floyd *et al.*, 1985). Benefits in terms of the enzyme assay are the determination of UDP from the feed, the presentation of reliable results within 1 to 4 hours, and no fistulated animals are required (Poos-Floyd *et al.*, 1985). It is postulated that tests with *Ficus glabrata*, *Aspergillus oryzae,* and *Corica papaya* obtained the best results (Poos-Floyd *et al.*, 1985). Nonetheless, more research is to be done relative to the use of bacteria and enzymes to predict potential protein degradability (Shannak *et al.*, 2000).

2.6 Procedures to increase the RUP fraction of feed

2.6.1 Extrusion (physical treatment)

Amino acids act as the building blocks of protein. Amino acids are utilized by the animal to sustain maximal growth, maintenance requirements, reproduction, and lactation (Limin & Rode, 1996). A broad spectrum of techniques or processes can be applied to increase the quality of amino

acids. Extrusion is a physical process (Esmail, 2019) that modifies the functional characteristics of feed ingredients by giving them a different structure. Extrusion causes fractional protein denaturation along with the occurrence of the Maillard reaction, allowing the proteins to be less susceptible to enzymatic attack by microorganisms in the rumen (Solanas *et al.*, 2008). The Maillard reaction consists of carbonyl groups of sugars combined with the free amino acids of proteins, due to the heat application (Kung & Rode, 1996), along with the formation of cross-linkages within and between peptide chains and carbohydrates (Deacon *et al.*, 1988). This technique is largely used in a variety of animal feed and food applications (Serrano *et al.*, 1998).

A summary of studies on the effect of extrusion on the rumen undegradable protein fraction (RUP) is presented in Table 2.1.

Table 2.1 Summary of studies on the effect of extrusion on the rumen undegradable protein fraction of different protein sources

Protein source	Increase in RUP-content	References
Lupins	34 %	(Limin & Rode, 1996)
Lupins and pea seed	9 %	(Aufrère *et al.*, 2001)
Faba beans	37 %	(Masoero *et al.*, 2005)
Canola oilcake meal	70 %	(Brand & Jordaan, 2020)
Crushed sweet lupins	108 %	(Brand & Jordaan, 2020)

Previous research pointed out that extrusion ultimately decreases protein degradability in the rumen and improves the nitrogen balance (Chalupa, 1975; Esmail, 2019). Van Straalen (1995) reported that heat treatment increased the fraction of undegradable protein and indigestible protein. This mainly depends on the duration of heat treatment and the temperature application. Overall, the enhancement of the intestinal protein digestibility of a feed source will result in increased animal performance in weight gain and feed conversion aspects (Chalupa, 1975; Konishi *et al.*, 1999). The temperature application of extrusion is crucial to prevent irreversible heat damage, making the protein indigestible to the animal (Solanas *et al.*, 2008). Extreme temperature conditions could result in decreasing the absorption of amino acids in the small intestines. It is stated that overheating could result in an 18% decrease in rumen protein digestibility (Esmail, 2019). Acid detergent insoluble nitrogen (ADIN) is used as an indicator of heat damage and an indication of the irreversible binding of amino acids (Chalupa, 1975). Thus, heat damage to feed must be avoided due to a resulting decrease in animal performance and production (Nakamura *et al.*, 1994). Previous research studies indicated that ideal extrusion conditions will result in a reduction in soluble N and a decrease in N associated with acid detergent insoluble nitrogen (ADIN) (McKinnon *et al.*, 1995). Masoero *et al.* (2005) indicated that extrusion improves starch enzymatic digestion of peas and faba beans by 27.9% and 73.7%, respectively, with lupins obtaining complete starch hydrolysis. Mendowski *et al.* (2019) stated that extrusion also efficiently decreases the effective degradability of the DM-fraction by approximately 20% in full-fat soybeans, full-fat canola seeds, soybean meal, canola meal, and sunflower meal (Konishi *et al.*, 1999). Additional research papers indicated the benefit of the

extrusion process in dairy feed, which led to an increase in UDP content and resulted in increased milk production per cow (Krizsan *et al*., 2017). Mendowski *et al.* (2019) similarly established an increase in milk yield of 2.6 kg/day/cow by providing a faba bean blend that was extruded at 140 °C.

Heating reduced the effective degradability of canola meal from 78.5% to 19.8%. Overall, it increased the feed efficiency in growing lambs by 32.4% (Plaisance *et al.*, 1997). Lastly, Serrano *et al*. (1998) confirmed that fully extruded feed enhanced the overall performance of calves by increasing their ADG by 14.3% (978 g/day *versus* 838 g/day) and decreasing the FCR by 20% (2.63 *versus* 3.29 kg feed/kg weight gain). Benchaar *et al*. (1994) also specified that extruded lupins increased amino acid movement to the main absorption site of cows by *ca* 34% and increased the absorption of amino acids in the small intestines of cows by *ca* 58%.

2.6.2 Formaldehyde (chemical treatment)

The value of feeding protein sources in ruminant nutrition is measured by how effectively the protein is degraded in the rumen and converted into microbial protein, as well as how effectively the bypass protein is digested in the small intestines (Subuh *et al.*, 1996). High-producing ruminants acquire high nutritional requirements to sustain their metabolic demands for production and performance. However, protein sources occasionally fall short in supplying the acquired amount of RUP and amino acids (Barry, 1976). Therefore, chemical treatments, specifically formaldehyde (HCHO) treatment, could be used to increase the RUP fractions of protein sources.

Gulati *et al*. (2005) stated that formaldehyde is a natural product of intermediary metabolism in mammals concerning the biosynthesis of amino acids. It is extensively used in both the food sector and animal feed manufacturing since it is a normal constituent in a variety of foods (Gulati *et al.*, 2005). Formaldehyde (HCHO) treatment consists of a series of chemical reactions with the protein and/or amino acids in the feed. This occurs under specific temperature and pH conditions. Formaldehyde is resistant to rumen breakdown at a pH of 5.5 - 7 (Barry, 1976; Mazinani *et al.*, 2020). The primary reaction occurs when a methylol compound is formed, followed by a slow condensation reaction taking place, where stable methylene cross-linkages are formed with the protein chains (Barry, 1976).

A summary of studies on the effect of formaldehyde treatment on either the nitrogen- or amino acid absorption in the small intestines (SI) against rumen degradation of a variety of protein sources are presented in Table 2.2.

Table 2.2 Summary of the effect of formaldehyde treatment on either the nitrogen- or amino acid absorption in the small intestines against rumen degradation of different protein sources

Protein source	Change in either nitrogen absorption and amino acid absorption in the small intestines	References
Herbage (before ensiling)	13% increase in AA absorption in small intestines	(Barry, 1976)
Canola	75% protein protection against rumen degradation (RUP of 300 g/kg)	(Gulati *et al*., 2005)
Soybean	77% protein protection against rumen degradation (RUP of 393 g/kg)	(Gulati *et al*., 2005)
Sunflower	73% protein protection against rumen degradation (RUP of 241 g/kg)	(Gulati *et al*., 2005)
Groundnut cake	70% increase in protein absorbed in small intestines	(Kumar *et al.*, 2014)

Thus, formaldehyde treatment may increase the amount of RUP and amino acids (Gulati *et al.*, 2005) due to the chemical reaction protecting the protein from rumen degradation, along with the methylene linkages being hydrolyzed in the abomasum in an acid-pepsin environment (Ferguson and Hemsley, 1967). A study by Van Straalen (1995) found that formaldehyde treatment significantly increased the RUP fraction of both canola meal and soybean meal. Formaldehyde treatment protects the protein source against rumen degradation by up to 75%, resulting in a 3:1 ratio of the RUP *versus* RDP fraction (Gulati *et al.*, 2005).

The formaldehyde treatment level is an essential aspect. Overprotection of HCHO will result in resistant linkages between formaldehyde and the reactive group of the protein, subsequently decreasing protein degradability in the small intestines (Gulati *et al.*, 2005). Previous studies indicated that the application of 10 g/kg CP (1%) formaldehyde attains a sufficient reduction in protein degradation and N solubility. No major effect was seen with increased formaldehyde concentrations during treatment.

A research study by Eghbali *et al.* (2011) stated that the formaldehyde treatment of a wide variety of different feed proteins exhibited a positive effect on the intestinal digestibility of protein, by decreasing the *in vitro* degradation rate of protein (Rodehutscord *et al.*, 1999) and the amount N retained by the animal (Kondusamy, 2010). Bhatt & Sahoo (2019) indicated that feeding HCHO-treated protein concentrate to post-weaned lambs resulted in improved protein digestion and overall live weight gain and feed conversion rate.

Formaldehyde treatment with the addition of sulphur-containing amino acids (SAA) could be used to enhance wool production. Rodehutscord *et al*. (1999) indicated that additional methionine supplementation along with HCHO-treated lupins fed to Merino wethers led to a 17% increase in wool growth compared to animals fed untreated lupins. It was also established that treatment of silage with HCHO increases overall animal production when fed to dairy cows, growing sheep, and cattle (Rodehutscord *et al.*, 1999). Beever *et al*. (1977) concluded that treatment of herbage with

HCHO before ensiling increased amino acid absorption from the small intestines by 13%. It also increased the amount of energy for metabolism by 1.3 MJ/kg of dry matter.

2.7 High-quality plant protein sources

2.7.1 Canola oilcake

Canola, also known as rapeseed (*Brassica napus L*.), is an oilseed plant, selected for relatively low levels of anti-nutritional factors such as erucic acid and glucosinolates (Heuzé *et al*., 2020). Also being a valued source of protein (*ca* 21%) and energy (*ca* 20.3 MJ/kg DM) (Blair, 2011) for the livestock feed industry, canola contains a high lipid content (*ca* 46%) and is abundant in polyunsaturated fatty acids (containing 21% linoleic and 10% linolenic acid) (Blair, 2011). Canola oilcake meal (CM) is also an excellent source of essential amino acids but is highly degradable in the rumen (Kendall *et al.*, 1991). Prior research indicated that the rumen degradation of CM varied from 44.3% (Kendall *et al.*, 1991) up to 74% (Moshtaghi Nia & Ingalls, 1995), thus being a perfect candidate feed source to apply treatment methods to increase the RUP.

In 2018, the main producers of canola meal were the EU (European Union) (12.8 million tons), followed by China (9.6 million tons), Canada (5.3 million tons), and India (4 million tons) (Heuzé *et al*., 2020). South Africa mainly imports canola and oilseed products due to undersupply of canola, relative to the domestic demand. The Protein Research Foundation (PRF) estimated South Africa's 2021 canola production to be 165 200 tons. The Department of Agriculture Forestry and Fisheries, (2017) indicated beef and sheep feed sales in 2016/2017 to be 3 090 591 tons. Raw material statistics stated that locally produced canola oilcake solely makes up 0.47% of the animal feed manufactured (27 304 tons) (Animal Feed Manufacturers Association (AFMA), 2021). Currently, the canola oilcake price is *ca* R9 811/ton (Protein Research Foundation, 2022).

A broad spectrum of canola cultivars is produced in South Africa (Department of Agriculture Forestry and Fisheries, 2016), with spring types dominantly being used. The largest canola harvest originates from the Southern Cape and the Western Cape, due to favorable climate conditions (Thomas, 2012). Canola has the benefit of reducing water runoff and soil erosion, due to its deep taproot system (Department of Agriculture Land Reform and Rural Development, 2021b). It was shown that canola production increased wheat production by *ca* 25% when used as a follow-up crop. Canola shows resistance against diseases and pests, thus reducing the manifestations of such elements in the soil. The protein value of untreated canola oilseed can be increased by a variety of processing methods (grinding, crushing, extrusion, and micronization) (Kim *et al*., 2001 Not in Ref. list).

2.7.2 Lupins

Lupins (*Lupinus*) is an annual legume plant that is utilized for seed and fodder, being a member of the *Fabaceae* family (Nwokolo & Smartt, 1996). Lupins are used globally in farming

systems as a rotational crop. Lupins ranked third in protein quality after soybean and chickpeas (Nwokolo & Smartt, 1996). The chemical composition of lupins is *ca* 40% CP on DM-basis, 13% fat, and 9% moisture (Nwokolo & Smartt, 1996). Lupins are also highly susceptible to rumen degradation (Sawaya *et al.*, 1984). The three main cultivars are blue lupin (*Lupinus angustifolius L*.), yellow lupin (*Lupinus luteus*) and white lupin L*lupinus albus*) (Heuzé *et al*., 2019). Blue lupin, also known as narrow-leaf lupin, is the dominant cultivar being farmed with, representing *ca* 91% of the total lupin production in Australia (ABARES & Pulse Australia Limited, 2016).

Lupinus angustifolius usually prefers neutral to acidic soil and does not exhibit resistance to drenched soil. It is a comparatively protein-rich source containing 31-38% CP on a DM basis (Nwokolo & Smartt, 1996), with a lysine content of 1.5% and methionine content of 0.22% (Bigalke *et al.*, 2016). It is generally used as a forage source for ruminants due to its high protein content. *Lupinus luteus* favours sandy to acidic soil and performs better in wet soil conditions in comparison to the other cultivars, with a crude protein content of *ca* 39-47% (Nwokolo & Smartt, 1996). *Lupinus albus* performs better in clay and sandy soil conditions (Agenbag, 1996), with a similar protein content as that of *Lupinus luteus* and a lysine and methionine content of respectively 1.8% and 0.27%.

Statistics of production in the Western Cape and Southern Cape regions of South Africa indicated an average yield (kg/ha) of 2 406 and 1 450 for *Lupinus albus* and L*upinus angustifolius* (Agenbag, 1996 Not in Ref. list), respectively. The Protein Research Foundation (PRF) estimated South Africa's 2021 sweet lupin production to be 29 700 tons (Protein Research Foundation, 2021), with full-fat lupins only used at a total of 0.01% in animal feed manufacturing (581 tons) (Animal Feed Manufacturers Association (AFMA), 2021). Currently, the sweet lupin price is *ca* R4 800/ton (Southern Oil PTY Ltd, 2022).

2.8 Background on sheep production in South Africa

In South Africa, 87.5% of available land can potentially be used for animal production. The remaining 12.5% is identified as arable land, suitable for crop production (Van Der Merwe *et al.*, 2020). The Department of Agriculture, Land Reform and Rural Development (2020) stipulated that the animal production sector in South Africa utilized 63,4 million hectares of land in 2019.

South Africa's sheep industry entails different sectors such as extensive and intensive sheep farming, feedlot finishing, and enhanced lambing systems (Van Der Merwe *et al.*, 2020a). Within the sheep industry, a diversity of different breeds are used, classified as wool, dual-purpose, and meat breeds. Wool and dual-purpose sheep breeds mainly consist of the Merino, South African Mutton Merino, and Dohne Merino. Meat breeds mainly consist of the Dorper, Dormer, and Meatmaster. Terminal sire breeds include the Suffolk, Ile De France, Dormer, and the Merino Landsheep. Indigenous breeds are mainly Afrikaner-type breeds, namely the Damara, Nguni, Pedi, and Swazi

(Soma *et al*., 2012). The sheep industry plays a major role in the regional context and rural parts of South Africa (Cloete *et al.*, 2014). Sheep numbers were estimated to *ca* 22 million in 2007, with sheep being the most abundant livestock species according to numbers (Cloete & Olivier, 2012). Overall, the gross value of the small stock (sheep and goats) produced in 2005/2006 was estimated to be R2 922 million (Cloete and Olivier, 2012) relative to R7 802 million in 2017/2018 (Department of Agriculture, Land Reform and Rural Development, 2020).

2.9 Meatmaster

The Meatmaster sheep breed is a modern synthetic breed, developed in South Africa in the early 1980's and was only registered as a breed in 2007 (Snyman, 2014). This breed is classified as a short haired fat-tailed breed, known for its hardiness and excellent fertility to perform under harsh extensive conditions. The breed was evolved using a fat-tailed Damara as the maternal line, bred with either the Dorper, South African Mutton Merino, Ile De France, or the Van Rooy breed (Van Der Merwe *et al.*, 2020a). The combination of breeds produced a well-balanced carcass conformation with good quality meat production achieved through low input costs. Thus, being an early maturing breed, the Meatmaster begins to deposit fat or adipose tissue earlier on. Van Der Merwe (2020) stated an ideal slaughter weight of 35.2 kg, with an ADG of 334 g/day and FCR of 4.21 kg feed/ weight kg gain for the breed.

The onset of earlier adipose tissue is theorized in being a result of elevated leptin blood levels which regulates intake by stimulating or suppressing appetite (Van Der Merwe *et al.*, 2020). Excess adipose tissue is predominantly in the tail of the Meatmaster sheep breed, thus, obtaining a carcass conformation with fat: lean tissue: bone ratio being 4:1:1. Desired premium carcass classification with the highest price obtained is A3/A2 followed by A4 classification, with Meatmaster lambs in the present study obtaining an overall carcass fat thickness score of 4 (7-9 mm). Nonetheless, the Red Meat Producers Organization of South Africa stated that approximately 72% of sheep slaughtered in commercial abattoirs are regarded as premium lamb (Van Der Merwe *et al.*, 2020). Snyman (2014) reported an average 100-day weaning weight of 27 kg for Meatmaster sheep, with rams and ewes achieving mature weights of 65 kg and 52 kg, respectively.

2.10 Precision lamb feedlot finishing

Meat & Livestock Australia defined a lamb finishing production system as being a system enhancing the animals' genetic potential through precision feeding and maintaining the health of lambs by feeding cost-efficient rations (Meat & Livestock Australia (MLA), 2005). The increasing mutton or meat demand steers farmers in the direction of increasing the biological efficiency and productivity of their sheep farming enterprises (Coetzee, 2013), with the objective of producing a greater number of earlier market-ready lambs. Sequentially, the market demands are met and profitability is sustained (Van Der Merwe *et al.*, 2020b). To maintain cost-efficiency within a feedlot operation, factors such as feeding costs, growth rates and efficiency (ADG; FCR), time of feed,

health management, and specific sheep genotype must be considered (Meat & Livestock Australia, 2005; 2017).

With nutrition being a major determinant of profitability, it is crucial to supply the sheep with a sufficient quantity of protein (amino acids), energy, fibre, bypass protein, vitamins and minerals for optimal genetic potential to be achieved (Coetzee, 2013). Feedlot lambs are fed high concentrated diets to achieve rapid growth rates in a shorter period (Beauchemin *et al.*, 1995). Coetzee (2013) defined precision feeding as feeding the correct supplement, at the precise quantity for a given period, based on the physiological stage of the animal. The National Research Council recommended a 14.50 %CP on a DM basis diet for early-weaned lambs to achieve a maximal growth rate (Beauchemin *et al.*, 1995). With lambs consuming approximately 3.80% to 4.20% of their body weight on a DM basis, the correct sheep breed is also important to achieve economic success. Crossbred lambs are favoured due to displaying faster growth rates, being early maturing, and obtaining higher prices per kg for their carcass (Duddy *et al*., 2019 2016 in Ref. list).

Due to different maturing rates of different sheep breeds, carcass characteristics differ in terms of carcass weight, carcass conformation, and carcass classification. In a feedlot study completed by Van Der Merwe *et al.* (2020b), late maturing breeds obtained higher carcass weight (20.7 kg), relative to earlier maturing breeds (16.9 kg). Ewe lambs also obtained a 1.90% higher dressing percentage relative to ram lambs, due to depositing fat at an earlier stage. An additional study concluded similar findings, with lighter weight lambs (40 kg live weight) entering the feedlot obtaining a greater growth rate (0.150 kg/day) and 12.3 kg weight gain (Meat & Livestock Australia, 2005), relative to heavier weight lambs (49 kg live weight) with a lower growth rate (0.110 kg/day) and 9 kg weight gain, with a similar feeding period. These results are explained due to heavier weight lambs achieving their mature growth stage at an earlier point, with earlier onset of adipose tissue.

2.11 Growth models

The description of growth curves in livestock is regarded as a necessity to improve or optimize feeding regimes, management practices, and genetic selection (Domínguez-Viveros *et al.*, 2019). Van Der Merwe *et al.* (2019) defined growth as the hyperplasia and hypertrophy of cells involving the tissue of an organism. The nutritional and environmental factors must be met, or the animal will not reach its full genetic potential, resulting in impaired performance. Thus, by optimizing the management feeding operation, increased production performance will be achieved (Abdelsattar *et al.*, 2021). Abdelsattar *et al.*, (2021) also stated that the transition period and development of the digestive tract of a rumen animal from birth up until post-weaning is important for the animal to fully mature and produce optimally. For production efficiency to be achieved within a lamb feedlot system, a minimum ADG of 0.300 kg/day must be maintained (Van Der Merwe *et al.*, 2019).

Empirical models or mechanistic approaches of internal mechanisms (Van Der Merwe *et al.*, 2019) can be used to determine the growth of sheep. However, the empirical models are preferred due to more accurate performance. The key empirical models entail the Brody $[\mathrm{Wt} = \mathrm{A}\ (1 - \mathrm{Be}^{-\mathrm{kt}})]$, Logistic $[\mathrm{Wt} = \mathrm{A}/(1 + \mathrm{Be}^{-\mathrm{kt}})]$, Gompertz $[\mathrm{Wt} = \mathrm{Ae}^{-\mathrm{e}-\mathrm{k}(\mathrm{t}-\mathrm{c})}]$ and Von Bertalanffy $[\mathrm{Wt} = \mathrm{A}(1 - \mathrm{Be}^{-\mathrm{kt}})^3$ models. Each model obtains its own functions, with Wt representing the body weight of the sheep at time t. A defines the asymptotic mature weight, B the amount of live weight gained after birth; k the maturation rate. Additionally, the Gompertz model's C represents the age at which maximum growth is reached (inflection point) (Van der Merwe *et al.*, 2019b).

In a recent study conducted by Van Der Merwe *et al.* (2019), the Logistic, Gompertz, and Von Bertalanffy were identified as being suitable for modeling the growth curve of a variety of South African sheep breeds, with the Logistic model being the most accurate in representing the growth curve of growing lambs. An additional study also identified the Gompertz model as a suitable model representing the body weight of the sheep; the Logistic model represents the carcass weight. Lambe *et al.* (2006) also selected the Gompertz model for its goodness of fit, compared to the Logistic and Richards models (Gbangboche *et al.*, 2008).

2.12 Voluntary feed intake (VFI) in small ruminants

Small ruminants (goats and sheep) are the third largest livestock group, contributing 11% of the total number of ruminants in the world (Pulina *et al.*, 2013). With the 2010 universal conversion index for small ruminants being 159 kg DM per kg protein produced (Pulina *et al.*, 2013), feeding is the main factor to reflect upon to achieve optimal production. For sustainability and profitability to be attained within a sheep feedlot operation, voluntary feed intake plays a vital role in the animals achieving maximum performance and growth. As stated by Knott *et al.* (2008), feed efficiency could be expressed by means of feed intake adjusted for live weight gain and live weight, or live weight gain adjusted for live weight and feed intake. Van Der Merwe *et al.* (2019) used the quadratic function DMI= AW^2+BW+C, where W is the body weight (kg) to model the feed intake of growing lambs up until maturity. Additionally, intake was also expressed as a percentage of body weight, with projected feed intakes being 4-5 % in growing lambs up to a 30 kg live weight and 2-3 % in lambs with a 70 kg live weight (Van Der Merwe *et al.*, 2019). This phenomenon is explained due to the growth of an animal in regards to its cumulative weight plotted against age, representing a sigmoidal curve. Their growth increases up to the point of inflection (at maximum body weight) and gradually decreases to the point where maturity is reached. Up to the point of inflection, the animal requires a greater amount of feed or nutrients for the development of muscles and tissue (Lewis & Emmans, 2010). After the point of inflection is reached, the onset of adipose tissue commences, and leptin hormones are secreted directly, decreasing feed intake (FI).

With regards to the Meatmaster sheep breed used, Van Der Merwe *et al.*(2019) stated that early maturing breeds obtained a 14% higher FI, relative to late maturing breeds, with the Meatmaster portraying an average FI of 1780 g/day at a live weight of 59.8 kg. Nonetheless, voluntary feed intake is influenced by a diversity of factors, such as animal factors, nutritional, environmental, genetic, and hormonal factors (Pulina e*t al*., 2013; Van der Merwe *et al.*, 2019). The live weight of an animal represents the animal factor. Nutritional factors involve the digestibility and gut fill characteristics of fibre in the diet, overall influencing the time spent ruminating and the passage rate (Pulina *et al.*, 2013). The feed intake is also dependent on neuropeptides, divided into stimulating appetite and suppressing appetite hormones, which are directly dependent on the secretion of leptin hormones *via* the adipose tissue. As previously discussed, the secretion of leptin hormones is associated with the energy balance of the animal. With excessive ingestion of carbohydrates or a high energy ration, FI decreases with the earlier onset of adipose tissue.

The end goal of a precision sheep feedlot system is to produce early market-ready carcasses, classified as premium carcasses with the correct composition (Van Der Merwe *et al.*, 2019). This is achieved through accurate feeding strategies and optimal feed utilization by the animal. Voluntary feed intake (VFI) is very important to determine the amount of feed required to attain the preferred slaughter weight.

2.13 Hypotheses

Thus, it is hypothesized that extrusion processing would potentially increase the amount of RUP in both locally produced plant protein sources, canola oilcake meal and sweet lupins, overall increasing the performance parameters of lambs under feedlot conditions.

Regarding the *in situ* trial, it is hypothesized that formaldehyde treatment will protect both plant protein sources, canola oilcake meal and sweet lupins, against rumen degradation and increase the amount of RUP and decrease effective rumen degradation, ultimately increasing the amount of RUP or bypass protein supplied to the small intestines for further absorption by the animal.

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Chapter 3

The effect of extrusion of canola oilcake meal and sweet lupins on the production performance of Meatmaster lambs under feedlot conditions

Abstract

Increasing commodity prices, cumulative export costs, diminishing use of genetically modified products and rivalry between the animal feed and the human food sector, present the opportunity to utilize processing techniques (such as extrusion) in order to fully optimize affordable, locally produced plant protein sources that cannot be used in human nutrition. Canola oilcake meal and sweet lupins were extruded in an attempt to increase the amount of rumen undegradable protein. In this study, Meatmaster lambs were kept under feedlot conditions and fed four different diets, namely canola oilcake meal control (CC), canola oilcake meal extrude (CE), sweet lupins control (LC) and sweet lupins extrude (LE), in order to determine the effect of extrusion processing of the two protein sources on their performance. No significant interaction was present for protein source and extrusion processing. Performance parameters such as average daily gain, feed conversion and feed intake were compared. A feed conversion ratio of 4.62 *versus* 4.85 kg feed/kg weight gain was present in the study for the extruded diet *versus* the control diet. An average daily gain of 0.310 kg/day and 0.320 kg/day were obtained for the control diet relative to the extruded diet. Similarly, no effect of extrusion on daily feed intake was observed between extruded (1.440 kg) and non-extruded (1.437 kg) protein sources. The production performance of lambs that received either canola oilcake meal (17.4% inclusion level) or sweet lupins (26.6% inclusion level) similarly did not differ significantly. An average fat score of 4 (7 to 9 mm back fat thickness) was achieved, with no effect of processing or protein source on the carcass fat thickness. Sweet lupins were identified as an inexpensive locally produced plant protein source, in comparison to canola oilcake meal, which obtains similar performance parameters in lambs under feedlot conditions in this study. Sweet lupins may be used as a good alternative to canola oilcake meal in well-balanced diets. With lupins being easily adaptable to poor soil and requiring less N fertilization, it is an ideal strategy to increase lupin production in South Africa.

3.1 Introduction

For 60 years, commercial extrusion processing has been actively used in the food and feed sector (Rahman *et al.*, 2015). Extrusion can be defined as a high temperature short time (HTST) process entailing heat and pressure to create a mechanical shear (Ministry of Primary Industries, 2021), which changes the texture and nutritional quality of the product. Along with an increasing competitiveness amongst the food and feed sector (Brand, 2007; Brand and Jordaan, 2020) and a decreasing popularity for the use of animal derived and genetically modified products in the feed

industry (Masoero *et al.*, 2006), the opportunity presented itself to explore affordable locally produced plant protein sources, which could be used as alternative protein source.

Canola oilcake meal and sweet lupins are identified in a variety of research studies as high quality protein sources that could be used in ruminant nutrition (Dickinson *et al*., 2004; Kriel, 2014; Brand & Jordaan, 2020). Canola oilcake is a good source of protein containing 30-35% CP on a DM-basis (Heuzé *et al*., 2020) and also containing a well-balanced amino acid profile, thus stimulating the growth of rumen microorganisms. Subsequently, the microorganisms are supplied with all the needed nutrients; a greater quantity of microbial protein is produced (Kriel, 2014), which results in better animal performance. Mustafa *et al.* (2003) stated that extrusion protects oilseeds and oilseed meals against rumen degradation, enhancing the quantity of amino acids available for intestinal digestion.

Lupins, specifically sweet lupins (*L..angustifolius*), are an additional less expensive high quality plant protein source, containing 37.5 % CP on a DM-basis (Brand, 2007). However, it is highly degradable in the rumen, resulting in insufficient rumen bypass protein (Brand & Jordaan, 2020) being supplied to the animal. Brand & Jordaan (2020) established that extrusion at 116˚C effectively decreases the effective rumen degradability of CP by 28%, without affecting the rate of degradation, resulting in potentially more sufficient use of lupins in the ruminant diet. Studies have proven that extrusion decreases ruminal degradability of legume seeds and oilcakes (Mustafa *et al.*, 2003). Claassen (2011) also stated that extrusion is a more affordable, environmentally friendly process that is energy-efficient and produces a smaller quantity of waste products relative to other heating processes. Extrusion processing could be applied to decrease rumen degradation; ultimately increasing the amount of bypass protein supplied to the small intestines for further absorption by the animal, resulting in better performance.

Thus, the aim in this study was to determine the effect of extrusion of canola oilcake meal and sweet lupins, and its effect on the performance of lambs under feedlot conditions. The effect of processing (extrusion) and protein sources on average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) performance parameters were determined in this study.

3.2 Materials and Methods

Ethical clearance for this study was granted by the Animal Care and Use Research Ethics Committee of Stellenbosch University (#21726) and DAEC (AP/NP/S/TB103) (Departmental Evaluation Committee) of the Agricultural Department, Western Cape Government at Elsenburg. Sixty-nine Meatmaster lambs at an average live weight of *ca* 22.9 kg, from Langgewens Research Farm, were used for this study. Lambs were housed at the metabolism facilities at Elsenburg, where

they were placed in individual paddocks (1.75m x 1.2m) each supplied with *ad libitum* feed and water.

Prior to the feeding trial, the lambs were preventatively treated for all internal and external parasites. A 7-day adaptation period was in place to ensure gradual adaptation of the rumen to the concentrated diet. During this period, the lambs were supplied with *ad libitum* chopped lucerne hay and 300 g pellets per lamb for the first three days. After the first three days, there were no restrictions to the concentrated pellets supplied to the lambs. Feeding was done twice a day, once in the morning (08:00) and in the afternoon (16:00). Each lamb was supplied with a specific concentrated feed, based on the treatment group allocated to them. Fresh feed and water were supplied on a daily basis.

The two tables below (Table 3.1 and Table 3.2) describes the diets fed during the feeding trial, being sweet lupin control (LC), sweet lupin extruded (LE), canola oilcake meal control (CC), canola oilcake meal extruded (CE). Lambs were randomly allocated to the treatment groups, with each group consisting of 16 lambs (7 ewes and 9 rams per group). Weekly weighing of lambs was done every Wednesday morning, when feed refusals were also weighed back, in order to determine weekly feed intake. The duration of the trial was approximately 4 months (from September up until January). When a live weight of *ca* 40 kg was reached (ideal slaughter weight of Meatmaster lambs is *ca* 35.2 kg to achieve an A2 grading (Van der Merwe *et al.*, 2019b), lambs were transported and slaughtered at DeliCo Abattoir in Riebeeck Kasteel, Western Cape, South Africa. Subsequently, a Mitutoyo Absolute Digimatic Caliper was used to measure the carcass fat thickness in millimeters.

The following concentrate formulated diets were fed during the experimental trial:

Table 3.1 Ingredients and nutrients for treatments (LC and LE)

Ingredients	**% As fed**
Maize	38.50 %
Sweet lupins	26.61 %
Wheat straw	25.75 %
Molasses powder	2.50 %
Bicarbonate of soda	2.00 %
Common salt, NaCl	1.00 %
Limestone, ground	1.00 %
Mono calcium phosphorus	0.64 %
Ammonium chloride	0.50 %
Ammonium sulphate	0.50 %
Slaked lime	0.50 %
Vitamin & Mineral Premix	0.50 %
Calculated nutrients composition	**% As fed**
TDN (Ruminants)	63.83 %
Crude protein	13.68 %
Crude fat	2.80 %
Crude fibre	18.01 %
Calcium	0.73 %
Phosphorus total	0.36 %

Table 3.2 Ingredients and nutrients for treatments (CC and CE)

Ingredients	**% As fed**
Maize	60.10 %
Canola oilcake meal	17.39 %
Wheat straw	14.05 %
Molasses powder	2.50 %
Bicarbonate of soda	2.00 %
Common salt, NaCl	1.00 %
Limestone, ground	0.96 %
Ammonium chloride	0.50 %
Ammonium sulphate	0.50 %
Slaked lime	0.50 %
Vitamin & Mineral Premix	0.50 %
Calculated nutrients composition	**% As fed**
TDN (Ruminants)	63.66 %
Crude protein	13.64 %
Crude fat	2.95 %
Crude fibre	9.23 %
Calcium	0.73 %
Phosphorus total	0.38 %

Results were statistically analyzed according to a 2 x 2 factorial design with extrusion and protein source as main factors, and sex used as block factor, using SAS 9.4 software (SAS Institute Inc., Cary, USA, 2016). The number of rams and ewes were balanced within each treatment to prevent gender effects. Mean values are accompanied by standard errors where applicable.

Canola oilcake meal and sweet lupins were purchased from SOILL in Moorreesburg, Western Cape, South Africa. Both these plant protein sources were extruded using an Insta Pro single screw extruder situated at Mariehndal experimental farm of the University of Stellenbosch. A single screw extruder was used, which is the renowned extruder used in producing livestock and aquatic feed, known for successfully converting mechanical energy to heat by means of friction (Rahman *et al.*, 2015). The material was heated to a specific temperature with a combination of moisture/steam and pressure, and exits the barrel as extrudates. The discharge pressure was between 30 - 60 atmosphere (Serrano, 1979). Pertaining to the experiment conducted, the extrusion conditions were at factor 30 for water, 17 for feed and a motor amp of 30 to 31, at a temperature of 113 ˚C.

Canola oilcake meal and sweet lupins were extruded along with Kalori 3000 (molasses powder), in order to obtain the Maillard reaction, also known as the browning effect, at a temperature of approximately 113 ˚C. After extrusion was completed, the extrudates were laid out on a net to dry and regularly turned over to prevent the possible occurrence of molding. This procedure was followed until a moisture content of 10-12% in the extruded feed was obtained. The moisture content of samples was measured utilizing a Moisture Analyzer MC2000. Thereafter, the extruded product was milled at Kromme Rhee Research Farm and used in producing the different formulated concentrate diets.

3.3 Results and Discussion

3.3.1 Statistical analysis of the production data revealed no significant interaction between extrusion and protein source. Processing (extrusion)

Table 3.3 The effect of extrusion of the protein sources on the production performance of Meatmaster lambs (means ± S.E.)

The effect of extrusion of the protein source on the production performance of Meatmaster lambs are presented in Table 3.3.

	Processing		Level of significance (*P*)
Parameters	**Not extruded**	**Extruded**	
Starting weight (kg)	22.64 ± 0.66	22.80 ± 0.66	0.86
End weight (kg)	35.30 ± 0.21	35.40 ± 0.21	0.72
Weight change (kg)	12.65 ± 0.66	12.60 ± 0.66	0.95
Days in feedlot (days)	43.41 ± 2.71	40.68 ± 2.72	0.48
ADG (kg/day)	0.300 ± 0.01	0.310 ± 0.01	0.58
Feed intake (kg/day)	1.43 ± 0.04	1.44 ± 0.04	0.96
FCR (kg feed/kg weight gain)	4.85 ± 0.17	4.62 ± 0.17	0.37

No significant differences in any of the measured production parameters were detected. These findings are in contrast to results obtained by (Serrano *et al*., 1998) where extruded feed, fed to calves during their transition phase (monogastric to ruminant), obtained an improved FCR relative to calves fed a control diet (2.63 vs 3.29). Similar feed intakes for both extruded feed (1.44 kg/day) and not extruded feed (1.43 kg/day) were found in this study. This result is supported by a study of Amirteymoori *et al.* (2021) on male Kermani lambs where the DMI of extruded linseed was comparable to the DMI of grounded linseed (1383 vs 1323 g/day).

Previous results (Brand & Jordaan, 2020) clearly indicated an increase in the RUP fractions of both sweet lupins and canola oilcake meal. These findings were not supported by the production results obtained in this study. Meatmaster lambs are an early maturing breed (Van der Merwe *et al.*, 2019a), indicating a possible lower requirement for bypass protein (van der Merwe *et al.*, 2020). Studies with late maturing breeds fed from an earlier age may present different results.

Lambs fed extruded feed obtained an ADG of 0.310 kg/day *versus* 0.300 kg/day obtained by lambs fed non-extruded feed. Comparable total weight gain throughout the trial for the extruded diet and control diet is present (12.60 kg *versus* 12.65 kg). Mendowski *et al*. (2019) similarly found no significant difference in the body weight or body condition score of dairy cows which were fed normal or extruded lupins. A lower body weight of lambs fed extruded linseed relative to grounded linseed (38.60 kg v*ersus* 40.40 kg) was observed by Amirteymoori *et al.* (2021).

Results found in this study may possibly be due to the extrusion conditions not being optimal in terms of temperature and duration of time spent in the extruder barrel. Deacon *et al.* (1988)

reported that the crude protein (CP) digestibility of protein sources extruded at 121˚C was not affected. Deacon *et al.* (1988) also suggested that insufficient response to extrusion may be due to too low extrusion temperatures and too short period of the material spent under pressure. Stated by both Serrano (1979) and Rahman *et al.* (2015), variation in extrusion results could possibly be dependent on a variety of factors, including type of extruders used, different extrusion conditions and also the particle size and composition of the feed being extruded. It is clear from the current results, as well as results from the literature, that the ideal extrusion conditions for different protein sources are not defined yet. The role and amount of sugar needed to provide the ideal conditions for the Maillard reaction to occur, without the irreversible binding of the protein sources, are similarly uncertain.

3.3.2 Protein sources

Table 3.4 The effect of protein sources (canola oilcake meal and sweet lupins) on the production performance of Meatmaster lambs (mean ± S.E.)

Table 3.4 presents the effect of protein sources (canola oilcake meal and sweet lupins) on the production performance of Meatmaster lambs.

	Protein source		Level of significance (*P*)
Parameters	**Canola oilcake meal**	**Sweet lupins**	
Starting weight (kg)	22.60 ± 0.64	22.84 ± 0.68	0.80
End weight (kg)	35.11 ± 0.21	35.58 ± 0.22	0.12
Weight gain (kg)	12.51 ± 0.64	12.74 ± 0.69	0.80
Days in feedlot (days)	41.79 ± 2.64	42.30 ± 2.81	0.89
ADG (kg/day)	0.310 ± 0.01	0.310 ± 0.01	0.81
Feed intake (kg)	1.39 ± 0.04	1.47 ± 0.04	0.16
FCR (kg feed/kg weight gain)	4.60 ± 0.17	4.87 ± 0.18	0.27

The feed intake of both canola oilcake meal and sweet lupins were relatively similar (1.39 vs 1.47 kg/day). A comparable number of days spent in the feedlot was represented by lambs fed both canola oilcake meal and sweet lupins (41.8 vs 42.3 days). No significant differences in FCR between lupins (4.87 kg feed/kg weight gain) and canola oilcake meal were observed. The results clearly indicate no significant differences in production performance of lambs either fed a diet with sweet lupins or canola oilcake meal as protein source.

In theory, canola oilcake meal is being classified as a moderate source of rumen undegradable protein (McKinnon *et al*., 1995), relative to sweet lupins being 81% degradable in the rumen (Mendowski *et al.*, 2019). Un-extruded canola oilcake meal supplies to some extent more microbial protein to the small intestines (Gous, 1998; Dickinson *et al.*, 2004; Kriel, 2014). In conclusion, differences between the protein source performance parameters were not significant (p <0.05), which indicates the potential of sweet lupins being used as potential protein source for lambs.

Table 3.5 Chemical composition of the treatment groups canola oilcake meal control (CC), canola oilcake meal extruded (CE), sweet lupins control (LC) and sweet lupins extruded (LE) fed to Meatmaster lambs under feedlot conditions

The chemical composition of the treatment groups CC, CE, LC, and LE are summarized in Table 3.5.

Treatment	DM (%)	Ash (%)	CP (%)	CF (%)	Fat (%)	NDF (%)	ADF (%)	ME (MJ/ kg)	In Vitro OMD (%)	TDN (%)	Ca (%)	P (%)
Canola oilcake meal												
Control	89.7	7.3	10.6	8.8	1.8	18.4	11.5	10.6	83.7	70.7	1.0	0.4
Extrude	89.6	8.2	10.4	9.4	1.5	17.4	12.0	10.3	85.7	69.3	1.1	0.4
Sweet lupins												
Control	90.5	8.7	9.9	13.8	1.9	25.9	17.2	10.1	86.4	67.9	1.2	0.4
Extrude	90.4	9.2	9.8	12.7	1.7	23.3	16.6	10.1	84.2	67.7	1.2	0.4

DM = Dry matter, CP = Crude Protein, CF = Crude Fibre, ND F= Neutral Detergent Fibre, ADF = Acid Detergent Fibre, ME = Metabolizable Energy, Ca = Calcium, P = Phosphorus, OMD = Organic Matter Digestibility, TDN = Total Digestible Nutrients

Extrusion did not seem to have an evident effect on the chemical composition of canola oilcake meal and sweet lupins either being extruded or not, regarding the CP content. Solanas *et al.* (2008) stated that extrusion has little effect on the chemical composition of feed mixtures.

3.3.3 Carcass fat thickness

The effect of processing and protein sources on the carcass fat thickness of Meatmaster lambs are presented in both Table 3.6 and Table 3.7.

Table 3.6 The effect of processing (extrusion) on the carcass fat thickness of Meatmaster lambs (mean ± S.E.)

Parameters	Processing		Level of Significance (*P*)
	Not Extruded	Extruded	0.55
Carcass fat thickness (mm)	7.52 ± 0.92	8.34 ± 1.02	

Table 3.7 The effect of protein sources (canola oilcake meal and sweet lupins) on the carcass fat thickness of Meatmaster lambs

Parameters	Protein Source		Level of Significance (*P*)
	Canola oilcake meal	Sweet lupins	0.26
Carcass fat thickness (mm)	8.72 ± 1.04	7.14 ± 0.89	

No significant differences ($p<0.05$) were observed for either protein sources (canola oilcake meal and sweet lupins) or processing (extrusion) on the carcass fat thickness of Meatmaster lambs.. The carcass fat thickness measurements of Meatmaster lambs that were fed canola oilcake meal (not extruded + extruded), sweet lupins (not extruded + extruded), extruded diets (canola + sweet lupins) and not extruded diets (canola + sweet lupins), were 8.72 mm ,7.14 mm, 8.34 mm and

7.52 mm, respectively. Regardless of processing, an average fat score of 4 (7-9 mm, with 11.6 to 14.6 % subcutaneous fat) (Van Der Merwe *et al.*, 2020a) was achieved during the feeding trial. This is due to the Meatmaster being an early maturing breed. Thus, the onset of adipose tissue starts earlier, relative to late maturing sheep breeds. This is a result of the elevated leptin blood levels, which regulates intake by stimulating or suppressing appetite (Van der Merwe *et al.*, 2019b). Plaisance *et al.* (1997) also stated that extrusion processing does not have any influence on carcass classification and composition.

Although there was no significant interaction between protein sources and extrusion processing, further improvement of performance parameters is possible by application of more precise extrusion conditions, in order to optimize the RUP fractions. Serrano (1979) stated that the processing conditions in previous research are not always well defined. Nevertheless, this research study has proven that similar performance parameters are achieved by using both canola oilcake meal and sweet lupins as alternative protein sources. With lupins costing R4 800 /ton (R1 278 contribution to diet costs in this study), relative to canola oilcake R9 264 /ton (R1 611 contribution to diet costs in this study) (Oil & Protein Seed Development Trust & Oilseed Advisory Comittee, 2022), the research study identified sweet lupins as a less expensive plant protein source, in comparison to canola oilcake meal, which obtains similar performance parameters in lambs under feedlot conditions. Availability and price of protein sources will ultimately determine the utilization in feed formulations (Brand, 2007). As pressure increases on the use of oilseed meals globally, this will stimulate lupin production in South Africa.

Future recommendations would be to test the precise temperature application and time period of raw materials spent in the extruder barrel. The addition of maize could possibly be a method to increase the stability of the extrudates, due to increasing gelatinization (Solanas *et al.*, 2008). Lastly, an alternative source of molasses powder could be exploited, in order to prevent possible crystallization in the extruder barrel, which ultimately delays the extrusion process.

3.4 Conclusion

With increasing commodity prices, animal feed protein sources becoming more expensive, and cumulative import prices, the need arises to explore more affordable locally produced plant protein sources which can be used as animal feed. No differences in production performance of Meatmaster lambs were found with regard to processing (extrusion) of either sweet lupins or canola oilcake meal in this study. Although previous results indicated a clear increase in RUP in protein sources due to extrusion, the time of feeding of the products in relation to the amino acid requirements of the animal at a specific stage may play an important role in results obtained. The ideal extrusion conditions and the amount of molasses added may also be critical, while certain processing conditions may include an irreversible binding of the amino acid in the protein source.

Nonetheless, sweet lupins are identified as a less expensive plant protein source, in comparison to canola oilcake meal, to be used in the diets of Meatmaster lambs under feedlot conditions in this growth stage. With lupins being easily adaptable to poor soil and requiring less N fertilization (Brand & Jordaan, 2020), it may be a potential alternative protein source for lambs in feedlots in South Africa.

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Chapter 4

The effect of formaldehyde treatment of canola oilcake meal and sweet lupins on the *in situ* dry matter and crude protein digestibility

Abstract

The value of feed protein sources in ruminant nutrition is measured by how effectively the protein is degraded in the rumen and converted into microbial protein, as well as how effectively the bypass protein is digested in the small intestines. High-producing ruminants have high nutritional requirements to sustain their metabolic demands for production and performance. However, protein sources occasionally fall short in supplying the acquired amount of rumen undegradable protein and amino acids. Chemical treatment (formaldehyde) could be used to increase the efficiency of protein sources, which are highly degradable in the rumen. Canola oilcake meal (CM) and sweet lupin seed (SL) were treated with formaldehyde (40% w/v) at concentrations of 10 g (F10) and 15 g/kg CP (F15). In this study, six Dohne Merino wethers fitted with rumen cannulas were used to determine the effect of formaldehyde treatment on the *in situ* dry matter and crude protein digestibility. The treatments entailed canola oilcake meal control (CMF0), canola oilcake meal treated with 10 g/kg CP formaldehyde (CMF10), canola oilcake meal treated with 15 g/kg CP formaldehyde (CMF15), sweet lupin seed control (SLF0), sweet lupin seed treated with 10 g/kg CP formaldehyde (SLF10) and sweet lupin seed treated with 15 g/kg CP formaldehyde (SLF15). Treatments were incubated in nylon bags in the rumen at time intervals of 0, 2, 4, 12, 36, 48, 72, and 96 hours. Formaldehyde significantly decreased the soluble fraction and lowered the rate of degradation of the potential degradable fraction at both concentrations, with plant protein sources CM and SL obtaining dry matter potential degradable fractions of 66.0% and 86.4%, respectively. The dry matter effective degradation was significantly decreased at all outflow rates (0.02, 0.04, 0.06, and 0.08/h) with the largest effect seen at 0.08/h. Both treatment concentrations (F10 and F15) decreased the dry matter effective degradation by 16.1% and 19.7%, respectively. Both formaldehyde treatments significantly increased the crude protein potential degradable fraction of CM by 11.8% and 32.1%, respectively. In contrast, F10 and F15 formaldehyde application decreased the crude protein potential degradable fraction of SL by 3.3% and 4.2%, respectively. Formaldehyde treatment also significantly decreased the crude protein effective degradation (43.8% at F10 and 38.1% at F15) at all outflow rates, with the largest effect seen at 0.08/h. Overall, formaldehyde treatment effectively decreased dry matter and crude protein rumen degradation at all outflow rates of both CM and SL. Therefore, formaldehyde treatment could be used to increase the rumen undegradable protein fraction. Potential improvement in animal performance in terms of live weight gain, average daily gain, and feed conversion efficiency has to be evaluated in production studies.

4.1 Introduction

Recently, emphasis has been placed on increasing high-producing ruminants' productivity by optimizing the animals' use of protein sources. Degradation models identified that amino acids absorbed by the animal mainly originate from the feed protein escaping rumen degradation and microbial protein (MP) (Poos-Floyd *et al.*, 1985). Kumar *et al.* (2015) stated that a variety of technical methods could be used to increase livestock productivity. Physical treatment (extrusion, grinding, and roasting) and chemical treatment (formaldehyde and alcohol application) could be used to increase the efficiency of certain protein sources, which are highly degradable in the rumen.

The formaldehyde reaction consists of two steps, entailing the rapid formation of a methylol compound, followed by a slow condensation reaction (Barry, 1976). Formaldehyde (HCHO) treatment reduces the activity of proteolytic bacteria on feed protein entering the rumen, by the formation of methylene cross-linkages between HCHO and the protein under ruminal pH conditions (Kumar *et al.*, 2014). The correct concentration of formaldehyde must be applied to prevent overprotection. Overprotection results in the methylene cross-linkages being irreversible as the protein enters the small intestines (SI). Sequentially, the amino acids will be unavailable to the animal, reducing the protein availability directed towards tissue growth and production. A variety of studies proved that 1% (10 g/kg CP) formaldehyde treatment is sufficient for the majority of feedstuffs (Malik *et al.*, 1981; Pratihar & Walli, 1995; Kondusamy, 2010). However, HCHO treatment differs between feedstuffs, being dependent on the protein solubility of the certain protein source (Barry, 1976).

Gulati *et al.* (2005) stated that HCHO treatment of a feedstuff has no harmful effect on the animal when ingested and no residue remains in the tissue of the animal (Wales *et al.*, 2009). However, human safety precautions must be in place regarding HCHO application, due to sensitivity of the human sensory system. Nonetheless, formaldehyde treatment is allowed as a feed processing method in the EU (Wales *et al.*, 2009). Previous research studies indicated that HCHO treatment decreases both *in situ* and *in vitro* effective ruminal degradation (Rodehutscord *et al.*, 1999) and protein degradation (Kumar *et al.*, 2014). Formaldehyde (HCHO) treatment along with methionine supplementation resulted in (*i*) increased wool growth (Rodehutscord *et al.*, 1999), (*ii*) increased protein digestion in the SI (Eghbali *et al.*, 2011), (*iii*) improved body weight gain (BWG), feed conversion ratio (FCR), and average daily gain (ADG), (*iv*) decreased *in vitro* ammonia concentration of fishmeal (Kondusamy, 2010), and (*v*) an overall increase of amino acid availability for further absorption in the SI (Barry, 1976; Bhatt & Sahoo, 2019). In addition, formaldehyde could be used as a fumigant gas (Wales *et al.*, 2009) to reduce or prevent antimicrobial infestations during feed storage, ultimately extending animal feed storage life.

Shannak *et al.* (2000) stated that a research gap pertaining trustworthy data based on the undegradable protein values (UDP) of concentrate ingredients exists The aim of this study was thus to determine the effect of formaldehyde treatment at levels of 0 g/kg CP, 10 g/kg CP, and 15 g/kg CP on the *in situ* dry matter and crude protein digestibility of locally produced plant protein sources, namely canola oilcake meal and sweet lupin seed.

4.2 Materials and Methods

4.2.1 Animals

Ethical clearance for this study was granted by the Animal Care and Use Research Ethics Committee of Stellenbosch University (#21726) and DAEC (AP/NP/S/TB103) (Departmental Evaluation Committee) of the Agricultural Department, Western Cape Government at Elsenburg. Six Dohne Merino wethers at a live weight *ca* 95 kg, already fitted with rumen cannulas, were housed at Kromme Rhee Experimental Farm of the Agricultural Department, Western Cape Government. Each animal was placed in its individual paddock (2.1 m x 2.0 m). Feeding was done twice a day, once in the morning (08:00) and the afternoon (16:00), along with fresh water being provided. They were fed an *ad libitum* basal diet consisting of 50:50 wheat straw and lucerne hay.

4.2.2 Treatments

The trial consisted of six treatments being tested *in situ*, based on the research of Orskov & Mcdonald (1979) on rumen protein degradability. The treatments entailed canola oilcake meal control (CMF0), canola oilcake meal treated with 10 g/kg CP formalin (40% w/v) (CMF10), canola oilcake meal treated with 15 g/kg CP formalin (40% w/v) (CMF15), sweet lupin seed control (SLF0), sweet lupin seed treated with 10 g/kg CP formalin (40 % w/v) (SLF10) and sweet lupin seed treated with 15 g/kg CP formalin (40% w/v) (SLF15).

The canola oilcake meal and sweet lupin seed (*Lupinus angustifolius*) were separately ground to 2 mm size, using a hammer mill (serial no 372), after which the ground samples were filtered using a Retch AS200 apparatus, to get rid of any powder that could potentially influence the dry matter (DM) disappearance. Afterwards, both canola oilcake meal and sweet lupins were separately placed in large zip lock bags at a 4 mm thickness level and sprayed with formalin (40 %w/v) (Kumar *et al.*, 2015). The bag was then vigorously shaken for 5 minutes before storage (Subuh & Rowan, 1994). The sprayed samples were left at room temperature for 24 hours for the reaction to occur (Antoniewicz *et al.*, 1992) and to prevent condensation of the formalin. After 24 hours, the samples were placed in tinfoil cups and retained in a force draught oven at 60 ˚C for 48 hours.

4.2.3 *In situ* evaluation of ruminal degradability

The dry matter (DM) and crude protein (CP) degradability of canola oilcake meal and sweet lupin seed were determined using the *in situ* technique described by Orskov & Mcdonald (1979). Both plant proteins were dried in a force draught oven for a minimum of 48 hours at 60 ºC. Afterwards, 5 g samples were weighed off in dacron bags (Brand & Jordaan, 2020) and tied off

using a constrictor knot with a nylon string, along with being colour coded using a cable tie, relative to which treatment it contained. For easy retrieval from the rumen, a washer was connected to the end of the string. The bags were incubated in the rumen at seven different time intervals.

The time intervals were 0, 2, 4, 12, 36, 48, 72 and 96 hours, with the 0-hour bag representing the control. The control bag was prepared identically to that of the other time intervals, except for not being placed in the rumen. It was also rinsed with tap water and placed in a force draught oven. The incubation period started with all seven bags (2, 4, 12, 36, 48, 72; 96 hours) being placed in the rumen every Monday morning at 07:00. The first bag retrieval was at 09:00 on a Monday morning, representing the 2-hour time interval, with the last bag being retrieved at 07:00 on Friday morning, representing the 96-hour bag. Treatments were randomly assigned to the six wethers making use of a cross-over design. Thus, each sheep received all six treatments, with each treatment being replicated six times (a total of 288 observations).

4.3 Chemical analysis

After retrieval from the rumen, the bags were rinsed under cold running tap water, to prevent further degradation, until the colour of the water draining from the dacron/nylon bag was clear. The bags were then placed in the oven at 60 ˚C for a minimum of 48 hours. Afterwards, the dried bags were weighed to determine the dry matter residue (Jordaan & Brand, 2020). The % nitrogen content of the residue was determined using a LECO TruMac N Nitrogen Determinator (LECO Corporation, Michigan, USA). The CP content of the dry matter was determined by multiplying the percentage N by a factor of 6.25. The rate of degradation was determined by the technique described by (Orskov & McDonald, 1979).

4.4 Statistical analysis

The DM and CP disappearances were stated as percentages of the amount of residue remaining after rumen incubation. The Mitscherlich function was used to fit the percentage of material degraded in the rumen, by means of SAS 9.4 software (SAS Institute Inc., Cary, USA, 2016) to determine the DM and CP degradability parameters:

$$Deg = A + B\,(1 - e^{-CT})$$

Deg represents the potential degradability at time, t (%), with A representing the rapidly soluble fraction (represents 0-hour disappearance (%)), B the fraction degraded over time (potentially degradable fraction (%)), and C the rate of degradation of the B fraction (%/h) (Jordaan & Brand, 2020).

With ruminal retention time affecting the degree of degradation, the fractional outflow rate of undegraded protein (UP) from the rumen (k) was considered in determining the percentage of

effective degradation. The following *k* values were used: 0.02 (low intake level), 0.04, 0.06 and 0.08/h (high intake level) (Brand & Jordaan, 2020). The percentage of effective degradation (Deg_{eff}) was determined by using a Latin square factorial design, with two protein sources (canola oilcake meal and sweet lupin seed) and three treatments (control / no formaldehyde application) (F0), 10 g/kg CP formaldehyde (F10) and 15 g/kg CP formaldehyde (F15) application). Thus, the percentage of effective degradation was determined using the equation:

$$Deg_{eff} = a + [b\,c\,/(c + k)]\,(1 - e^{-(c+k)\,t})$$

4.5 Results and Discussion

No interaction was present for the dry matter soluble fraction (*A*), dry matter potential degradable fraction (*B*), and the rate of degradation of *B* (*C*), for protein source (CM & SL) and processing (formaldehyde treatment).

Significant differences were present for the DM soluble fraction (*P*=0.036) and the rate of degradation of the potential degradable fraction estimates for the DM degradability (*P* <.0001), with processing as the main factor. Significant differences were also present for all the DM non-linear parameters (*P* <.0001) for protein source as the main factor. Thus, sweet lupin seed obtained a 23.6% (86.4% *versus* 66.0%) higher DM potential degradable fraction in the rumen of the sheep, relative to canola oilcake meal. These results were supported by Heuzé *et al.*, 2020 and Heuzé *et al.*, 2022), where the average potential degradable fraction of *Lupinus angustifolius* was 5% higher compared to canola oilcake meal (60% *versus* 55%).

The *in situ* results of the DM disappearance parameters for the effect of formaldehyde treatment on plant protein sources, namely canola oilcake meal (CM) and sweet lupin seed (SL), are summarized in Table 4.1.

Table 4.1 The effect of formaldehyde (HCHO) treatment on the mean (± S.E.*) in situ* dry matter (DM) rumen disappearance of non-linear parameters of canola oilcake meal and sweet lupin seed

		*Dry matter non-linear parameters		
		A	***B***	***C***
Protein source	Canola oilcake meal (CM) Sweet lupin seed (SL) *P* value	10.1^{a} ± 2.6 7.2 ^{b} ± 2.1 <.0001	66.0^{a} ± 3.7 86.4^{b} ± 3.4 <.0001	0.07^{a} ± 0.05 0.11^{b} ± 0.05 <.0001
Processing	Control (F0) HCHO treatment 10 g/kg CP (F10) HCHO treatment 15 g/kg CP (F15) *P* value	7.6^{b} ± 2.5 9.3^{a} ± 3.2 9.1^{a} ± 2.4 0.036	76.6^{a} ± 10.7 76.0^{a}± 11.7 76.1^{a} ± 11.4 0.911	0.15^{a} ± 0.03 0.06^{b} ± 0.03 0.05^{b} ± 0.02 <.0001
Protein source x Processing	CM control (CMF0) CM treated with 10 g/kg CP HCHO (CMF10) CM treated with 15 g/kg CP HCHO (CMF15) SL control (SLF0) SL treated with 10 g/kg CP HCHO (SLF10) SL treated with 15 g/kg CP HCHO (SLF15) *P* value	8.5^{b} ± 2.5 10.8^{a}± 3.2 11.1^{a}± 1.5 6.7^{b}± 2.5 7.8^{b}± 2.6 7.1^{b}± 1.1 0.285	67.0^{b} ± 2.04 65.2^{b} ± 3.9 65.9^{b}± 5.1 86.2^{a} ± 4.9 86.8^{a} ± 2.6 86.3^{a} ± 3.1 0.740	0.13^{b} ± 0.03 0.05^{d}± 0.02 0.03^{d}± 0.01 0.18^{a}± 0.01 0.08^{c}± 0.03 0.06^{c}± 0.01 0.428

**A*= rapidly soluble fraction (%), *B*= the fraction that will degrade over time (%), *C*= the rate of degradation of the *B* fraction (%/h)

a,b,c Denote significant differences (*P* <0.05) in columns

The dry matter effective degradability (Deg_{eff}) for all treatments at various outflow rates are presented in Table 4.2. Significant differences were present for processing at all outflow rates.

Table 4.2 The effect of formaldehyde (HCHO) treatment on the mean (± S.E.*) in situ* dry matter (DM) effective degradation from the rumen of canola oilcake meal and sweet lupin seed

		Dry matter effective degradation at fractional outflow rate (%)			
		0.02/h	**0.04/h**	**0.06/h**	**0.08/h**
Protein source	Canola oilcake meal (CM) Sweet lupin seed (SL) *P* value	56.8^{a}± 7.14 77.8^{b}± 5.5 <.0001	47.4^{a} ± 9.05 67.4^{b} ± 8.0 <.0001	41.4^{a}± 9.4 59.8^{b} ± 9.15 <.0001	37.3^{a} ± 9.2 54.1^{b}± 9.6 <.0001
Processing	Control (F0) HCHO treatment 10 g/kg CP (F10) HCHO treatment 15 g/kg CP (F15) *P* value	75.1^{a} ± 9.3 65.0^{b} ± 11.2 61.9^{c} ± 12.6 <.0001	68.0^{a} ± 9.7 53.9^{b} ± 10.9 50.2^{c} ± 12.1 <.0001	62.3^{a} ± 0.8 46.7^{b} ± 10.2 42.9^{c} ± 10.9 <.0001	57.6^{a} ± 9.8 41.5^{b} ± 9.4 37.9^{c} ± 9.7 <.0001

a,b,c Denote significant differences (*P*<0.05) in columns

As seen, formaldehyde treatment at both concentrations of 10 g/kg CP and 15 g/kg CP effectively decreased the DM effective degradation at each fractional outflow rate, with the largest effect seen at 0.08/h (from 57.6/h to 37.9/h). These results are supported by Eghbali *et al.* (2011) where canola oilcake meal treated with 12 g/kg CP HCHO effectively decreased the Deg_{eff} by 21.2% at an 0.08/h outflow rate. It is suggested that formaldehyde application enhances the apparent digestibility of protein (Eghbali *et al.*, 2011). Thus, the F10 treatment decreased the DM effective degradation by 10.1%, 14.1%, 15.6% and 16.1% at outflow rates of 0.02, 0.04, 0.06, and

0.08/h, respectively. Additionally, the F15 treatment decreased DM effective degradation by 13.2%, 17.8%, 19.4% and 20.0% at outflow rates of 0.02, 0.04, 0.06, and 0.08/h, respectively. Significant differences were also present at each fractional outflow rate for both protein sources, canola oilcake meal and sweet lupin seed (*P*<.0001).

Figure 4.1 and 4.2 illustrates that formaldehyde treatment at concentrations of 10 g/kg and 15 g/kg CP, effectively decreases the DM disappearance of both canola oilcake meal and sweet lupin seed at different rumen incubation time intervals (0, 2, 4, 12, 36, 48, 72, and 96 hours).

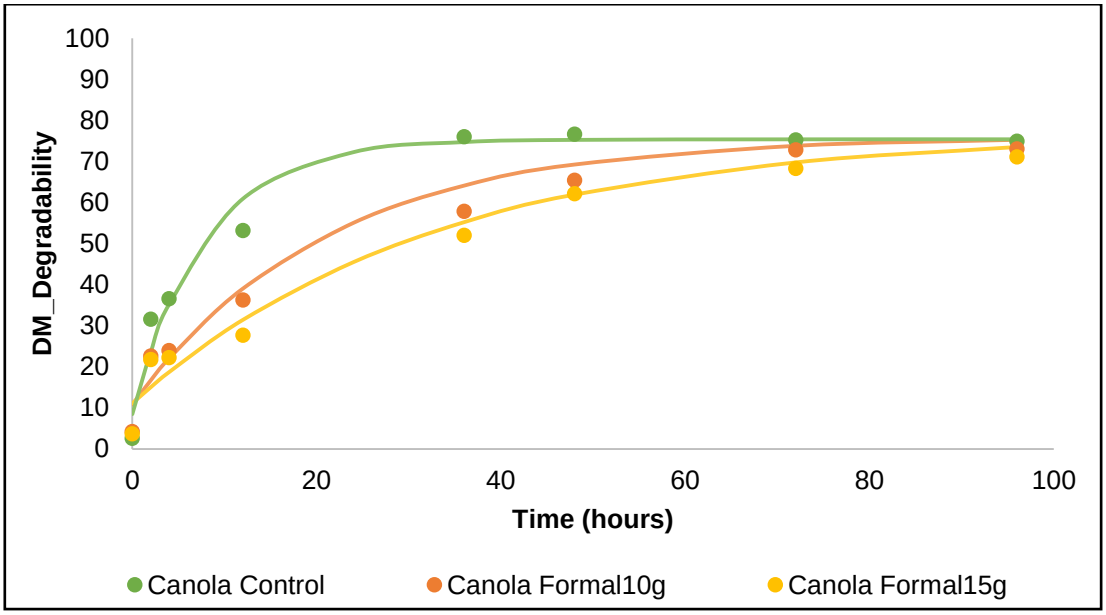

Figure 4.1 The effect of formaldehyde treatment on the DM degradability of canola oilcake meal at different rumen incubation time intervals

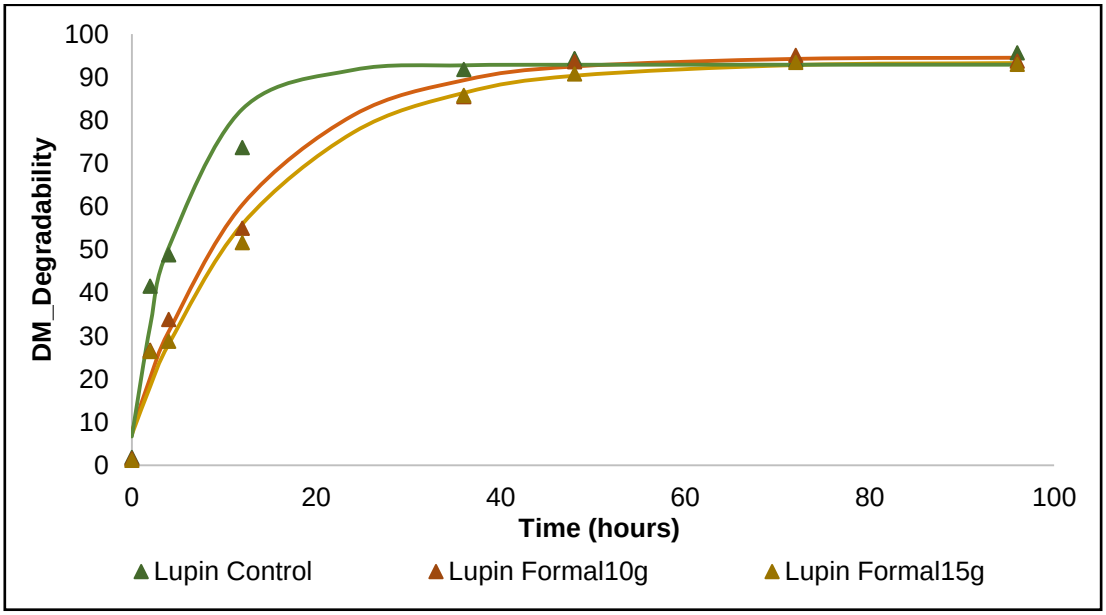

Figure 4.2 The effect of formaldehyde treatment on the DM degradability of sweet lupin seed at different rumen incubation time intervals

The *in situ* results of the CP disappearance parameters for the effect of formaldehyde treatment on plant protein sources, canola oilcake meal (CM) and sweet lupin seed (SL), are summarized in Table 4.3.

Table 4.3 The effect of formaldehyde (HCHO) treatment on the means (± S.E.) *in situ* crude protein (CP) rumen disappearance of non-linear parameters of canola oilcake meal and sweet lupin seed

		*Crude protein non-linear parameters		
		A	***B***	***C***
Protein source	Canola oilcake meal (CM)	$10.6^{a} \pm 0.4$	$93.43^{a} \pm 1.0$	$0.05^{a} \pm 0.01$
	Sweet lupin seed (SL)	$16.9^{b} \pm 0.3$	$80.72^{b} \pm 0.9$	$0.2^{b} \pm 0.005$
	P value	<.0001	<.0001	<.0001
Pro-cessing	Control (F0)	$14.0^{a} \pm 1.7$	$81.0^{c} \pm 2.9$	$0.3^{a} \pm 0.2$
	HCHO treatment 10 g/kg CP (F10)	$13.6^{a} \pm 4.7$	$85.3^{b} \pm 6.6$	$0.05^{b} \pm 0.03$
	HCHO treatment 15 g/kg CP (F15)	$14.9^{a} \pm 5.9$	$91.4^{a} \pm 17.1$	$0.04^{b} \pm 0.01$
	P value	0.441	<.0001	<.0001
Protein source x Pro-cessing	CM control (CMF0)	$14.2^{c} \pm 2.1$	$78.8^{c} \pm 2.03$	$0.1^{b} \pm 0.03$
	CM treated with 10 g/kg CP HCHO (CMF10)	$9.7^{d} \pm 2.2$	$90.6^{b} \pm 4.9$	$0.03^{d,e} \pm 0.01$
	CM treated with 15 g/kg CP HCHO (CMF15)	$8.3^{d} \pm 0.7$	$109.9^{a} \pm 9.2$	$0.02^{e} \pm 0.01$
	SL control (SLF0)	$13.9^{c} \pm 1.5$	$83.2^{c} \pm 1.6$	$0.5^{a} \pm 0.04$
	SL treated with 10 g/kg CP HCHO (SLF10)	$17.5^{b} \pm 2.7$	$79.9^{c} \pm 2.3$	$0.1^{c} \pm 0.03$
	SL treated with 15 g/kg CP HCHO (SLF15)	$19.2^{a} \pm 2.5$	$79.0^{c} \pm 4.0$	$0.05^{c,d} \pm 0.01$
	P value	<.0001	<.0001	<.0001

**A*= rapidly soluble fraction (%), *B*= the fraction that will degrade over time (%), *C*= the rate of degradation of the *B* fraction (%/h)

a,b,c,d;e Denote significant differences (*P*<0.05) in columns

It is evident that an interaction is observed for the soluble fraction, potential degradable fraction, and the rate of degradation of the potential degradable fraction (*P* <.0001). Therefore, the main effects cannot be interpreted. Formaldehyde treatment at both concentrations (F10 and F15) decreased the soluble fraction of canola oilcake meal but increased the soluble fraction in sweet lupin seed. Formaldehyde treatment increased the potential degradable fraction of canola oilcake meal by 11.8% (at F10) and 32.1% (at F15). These results are similar to results presented by Subuh *et al.* (1994) where canola oilcake meal treated with 8 g/kg CP HCHO (30% formalin) decreased the ruminal degradation by 11.2 %. Additionally, Eghbali *et al.* (2011) suggested that canola oilcake meal treated with 12 g/kg CP HCHO led to a 30.7% decrease in the soluble fraction, 11.9% increase in potential degradable fraction, and a 43.5% decrease in the rate of degradation of the potential degradable fraction. Furthermore, formaldehyde treatment decreased the rate of degradation of the CP potential degradable fraction of both canola oilcake meal and sweet lupin seed by 70% (CMF10), 80% (CMF15), 80% (SLF10), and 90% (SLF15), respectively.

However, formaldehyde treatment decreased the potential soluble fraction of sweet lupin seed by 3.3% (at F10) and 4.2% (at F15).The nitrogen digestibility of *Lupinus angustifolius* is 2.8% higher compared to canola oilcake meal (80% *versus* 77.2%) (Heuzé *et al.*, 2020 & 2022). Thus, the above result could potentially be explained due to protein solubility influencing the potential

degradable protein fraction. It is, therefore, probable that 10 g/kg CP and 15 g/kg CP HCHO treatment of the sweet lupin seed exceeded the optimal level of HCHO treatment. Thus, overprotection resulted in a slight decrease in the potential degradable fraction due to complexes formed between HCHO and the protein group, overall rendering sweet lupin seed less digestible, decreasing the protein digestibility (Gulati *et al.*, 2005 & Kumar *et al.*, 2015).

Table 4.4 The effect of formaldehyde (HCHO) treatment on the mean (± S.E.) *in situ* crude protein (CP) effective degradation from the rumen of canola oilcake meal and sweet lupin seed

		Crude protein effective degradation at fractional outflow rate (%)			
		0.02/h	**0.04/h**	**0.06/h**	**0.08/h**
Protein source	Canola oilcake meal (CM) Sweet lupin seed (SL) *P* value	65.5^{a} ± 0.6 83.0^{b}± 0.6 <.0001	51.7^{a} ± 0.8 74.1^{b}± 0.7 <.0001	44.0^{a} ± 0.8 68.0^{b}± 0.8 <.0001	39.0^{a} ± 0.9 63.4^{b} ± 0.8 <.0001
Pro-cessing	Control (F0) HCHO treatment 10 g/kg CP (F10) HCHO treatment 15 g/kg CP (F15) *P* value	86.8^{a} ± 7.2 70.3^{b} ± 9.6 67.7^{c} ± 11.7 <.0001	80.7^{a} ± 10.4 57.1^{b} ± 12.2 53.7^{c} ± 13.8 <.0001	75.7^{a} ± 12.5 49.2^{b} ± 12.2 45.8^{c} ± 13.6 <.0001	71.7^{a} ± 13.9 43.8^{b} ± 12.6 40.6^{c} ± 13.1 <.0001

^{a,b,c} Denote significant differences (*P* <0.05) in columns

The crude protein effective degradability (Deg_{eff}) for all treatments at various outflow rates is presented in Table 4.4. Significant differences were present for processing at all outflow rates, 0.02, 0.04, 0.06, and 0.08/h. Formaldehyde treatment at both concentrations of 10 g/kg CP and 15 g/kg CP formaldehyde (HCHO) effectively decreased the CP effective degradation at each fractional outflow rate, with the largest effect seen at 0.08/h (from 71.7% to 38.1%). Thus, F10 concentration treatment decreased CP effective degradation by 16.5%, 23.6%, 26.5% and 27.9%, at outflow rates of 0.02, 0.04, 0.06 and 0.08/h, respectively. Additionally, the F15 concentration treatment decreased CP effective degradation by 19.1%, 27%, 29.9% and 31.1%, at outflow rates of 0.02, 0.04, 0.06 and 0.08/h, respectively. Significant differences were also present at each fractional outflow rate for both protein sources, canola oilcake meal and sweet lupin seed (*P*<.0001). This is supported by results obtained by Rodehutscord *et al.* (1999) where lupins treated with 4 g/kg CP HCHO significantly decreased the fractional outflow rate of nitrogen disappearance at a 0.03/h outflow rate.

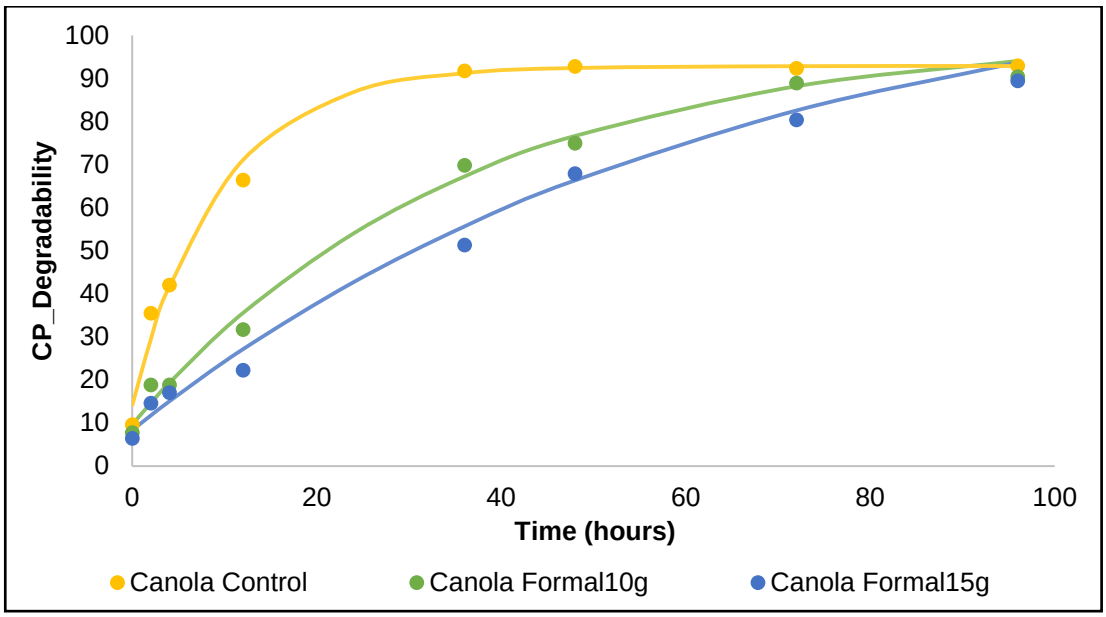

Figure 4.3 The effect of formaldehyde treatment on the CP degradability of canola oilcake meal at different rumen incubation time intervals

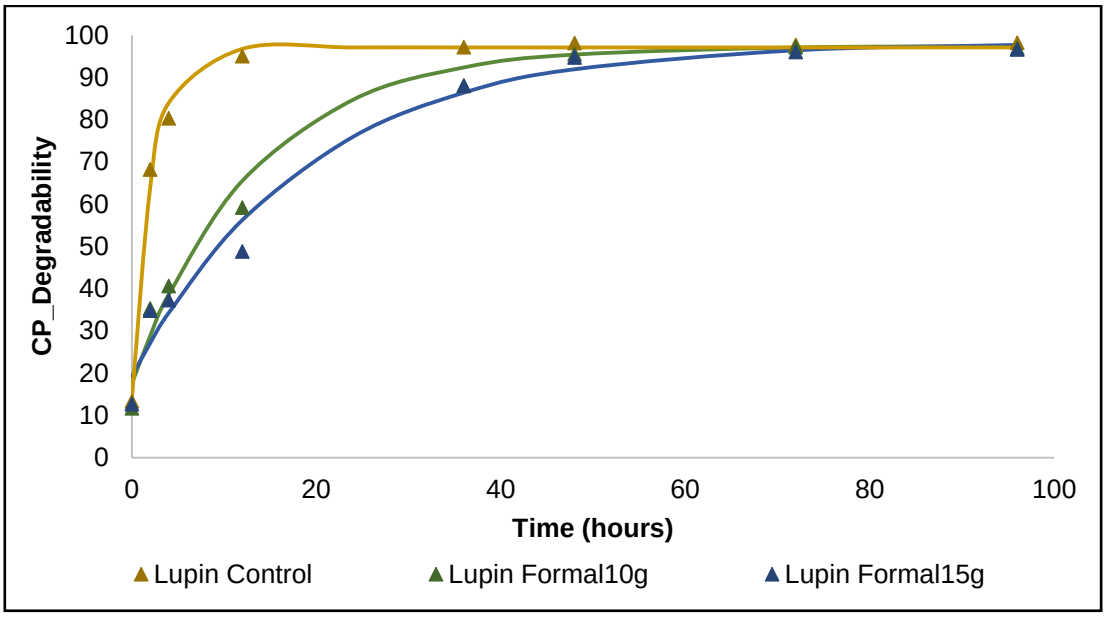

Figure 4.4 The effect of formaldehyde treatment on the CP degradability of sweet lupin seed at different rumen incubation time intervals

Figure 4.3 and 4.4 illustrates that formaldehyde treatment at both concentrations of 10 g/kg CP and 15 g/kg CP HCHO, gradually decreases the CP degradability of both canola oilcake meal and sweet lupin seeds at different rumen incubation time intervals (0, 2, 4, 12, 36, 48, 72, and 96 hours).

A number of previous studies suggested that formaldehyde (HCHO) treatment of different protein sources supports increased live weight gain (LWG) and improved feed conversion efficiency (Peter *et al.*, 1971; Spears *et al.*, 1980; Bhatt & Sahoo, 2019) in ruminants. (Gupta & Gupta, 2012; Chopra *et al.*, 2013) also found similar results obtained at a 10 g/kg CP HCHO application level, compared to a 20 g/kg CP HCHO application that drastically decreased LWG and

impaired FCE. Additionally, Kondusamy (2010) indicated that 10 g/kg CP HCHO application significantly decreased the nitrogen solubility and *in vitro* ammonia levels of sardine fishmeal. With formaldehyde being a product of intermediate metabolism in mammals (Gulati *et al.*, 2005), it is an effective strategy and feasible technology (Kumar *et al.*, 2014) to increase the rumen undegradable protein or bypass protein of protein sources which is highly degradable in the rumen. Nonetheless, optimal HCHO application is crucial, to optimize the quantity and quality (Bhatt & Sahoo, 2019) of the protein available in the small intestines of the ruminant.

4.6 Conclusion

Processing (formaldehyde treatment) at both 10g and 15g/kg CP concentrations, significantly increased the rapidly soluble fraction and lowered the rate of degradation of the DM potential degradable fraction (*P* <.0001), with plant protein sources CM and SL obtaining potential degradable fractions of 66.0% and 86.4%, respectively. Formaldehyde treatment at both concentrations significantly decreased effective DM degradation (Deg_{eff}) at all outflow rates with the largest effect seen at 0.08/h. Formaldehyde 10 g/kg CP treatment decreased the DM Deg_{eff} by 16.1% and formaldehyde 15 g/kg CP treatment decreased DM Deg_{eff} by 19.7% (at an outflow rate of 0.08/h).

Formaldehyde treatment at both concentrations significantly increased the crude protein (CP) potential degradable fraction of CM by 11.8% (F10) and 32.1% (F15), respectively. In contrast, F10 and F15 formaldehyde application decreased the potential degradable fraction of SL by 3.3% and 4.2%, respectively. Nonetheless, HCHO treatment significantly decreased (*P* <.0001) CP Deg_{eff} at all outflow rates, with the largest effect seen at 0.08/h. Formaldehyde 10 g/kg CP treatment decreased the CP Deg_{eff} by 27.9% and formaldehyde 15 g/kg CP treatment decreased CP Deg_{eff} by 31.1%.

To conclude, formaldehyde (HCHO) treatment effectively decreases both DM and CP rumen degradation at all outflow rates of both CM and SL. Therefore, HCHO application could be used to increase the rumen undegradable protein fraction of highly degradable protein sources. Potential improvement of animal performance in terms of live weight gain, average daily gain, and feed conversion efficiency should be tested in practice.

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Chapter 5

General conclusions and future recommendations

The research project aimed to utilize techniques (physical and chemical treatment) to potentially increase the rumen undegradable protein fractions (RUP) of locally produced plant protein sources (canola oilcake meal (CM) and sweet lupin seed (SL)). The applied methods entailed physical treatment, namely extrusion at *ca* 113ºC with the addition of 6% molasses (Trial 1), and chemical treatment, namely, formaldehyde treatment (40% w/v formalin) at concentrations of 0 g, 10 g, and 15 g/kg CP (Trial 2).

5.1 Conclusions per objective

- With regards to Trial 1 (Chapter 3), no differences in production performance of Meatmaster lambs used in this study were found with regard to extrusion (processing) of either the sweet lupins or canola oilcake meal (*P* >0.05).
- Lambs that consumed a diet with sweet lupins as a protein source, however, produced at the same level as lambs that consumed a diet with canola oilcake meal as protein source.
- Sweet lupins (R4800/ton) (R1278 contribution to diet costs in this study) were identified as a less expensive plant protein source, in comparison to canola oilcake meal (R9264/ton) (R1611 contribution to diet costs in this study), to use in the diets of lambs under feedlot conditions.
- Lupins may potentially be used as an alternative protein source for lambs in feedlots in South Africa.
- With regard to Trial 2 (Chapter 4), formaldehyde treatment at both concentrations of 10 g and 15 g/kg CP decreased the soluble fraction and lowered the rate of degradation of the potential degradable fraction in both plant protein sources (CM and SL) (*P*<.0001).
- Formaldehyde treatment at both concentrations significantly decreased the effective DM degradation (Deg_{eff}) at all outflow rates with the largest effect seen at 0.08/h. Thus, formaldehyde decreased DM Deg_{eff} by 34.2% and 28.3% at 10 g and 15 g/kg CP concentrations, respectively.
- Formaldehyde treatment at both concentrations (F10 and F15) significantly increased the potential degradable fraction of CM by 13.0% and 28.3%, respectively.
- Overall, formaldehyde application significantly decreased the effective CP degradation of both lupin seeds and canola oilcake meal at all outflow rates, with the largest effect seen at 0.08/h. Thus, formaldehyde decreased CP Deg_{eff} of lupin seeds and canola oilcake meal by 43.4% and 38.9% at 10 g and 15 g/kg CP concentrations, respectively.

It is important to test the observed positive results with the application of formaldehyde treatment of lupin seeds and canola oilcake meal in practice with animals in feedlots.

5.2 Future prospects

With increasing lamb and mutton prices, animal feed prices, and rivalry between the food and feed sectors affecting the profitability within a farming operation, farmers are driven towards intensifying their sheep finishing systems to produce early market-ready lambs. Thus, with enduring increases in overall farming operational costs, the opportunity is presented in identifying alternative, more affordable, locally produced plant protein sources which are easily accessible. Processing methods such as extrusion (physical treatment) and formaldehyde application (chemical treatment) could potentially be used to increase the protein availability of alternative plant protein sources, which are highly degradable, thus increasing the rumen undegradable protein fraction.

Research indicated a gap in determining the specific extrusion conditions, per individual plant protein source, to ensure optimal physical treatment and prevent heat damage or overprotection, which could alter the production performance of the animal. An alternative form of molasses (excluding molasses powder), which could potentially be extruded together with the plant protein source, must also be trialed, ultimately enhancing the Maillard effect, however, preventing crystallization of the extrudates.

With regards to formaldehyde application, further research is required in terms of a growth trial, to determine the effect of formaldehyde treatment of canola oilcake meal and sweet lupins on the production performance of lambs under feedlot conditions. In Chapter 4, formaldehyde treatment at concentrations of 10 g and 15 g/kg CP displayed promising results, and should thus be explored.

In conclusion, the current dissertation provides farmers with information on alternative plant protein sources (canola oilcake meal and sweet lupins), with sweet lupins obtaining similar performance parameters in lambs under feedlot conditions, relative to canola oilcake meal. Formaldehyde application at concentrations of 10 and 15 g/kg CP effectively decreased rumen degradation of both canola oilcake meal and sweet lupins, thereby providing research information that could be used to enhance optimal utilization of plant protein sources, potentially improving animal performance.