Effect of protein source and quality on feedlot growth performance and rumen fermentation characteristics of Döhne Merino lambs

By

Ashley Grimsell

Submitted in fulfilment of the requirements for the degree

MSc (Agric) Animal Science: Animal Nutrition

Department of Animal and Wildlife Sciences

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

February 2020

Table of Contents

Declaration i
Acknowledgements ii
Summary iii
Abbreviations v
Abbreviations of Amino Acids viii
List of Tables ix
List of Figures x
Chapter 1 1
Introduction and Motivation 1
Hypotheses 3
Chapter 2 4
Literature Review 4
2.1 Overview of sheep feedlots 4
2.2. Dried brewers grain 5
2.2.1 Nutrient characteristics of dried brewers grain 5
2.2.2 Effect of dried brewers grain supplementation on performance of feedlot sheep and cattle 7
2.3. Canola meal 9
2.3.1 Nutrient characteristics of canola meal 9
2.3.2 Effect of canola meal supplementation on performance of feedlot sheep and cattle 13
2.4 Soybean meal 14
2.4.1 Nutrient characteristics of soybean meal 14
2.4.2 Effect of soybean meal supplementation on performance of feedlot sheep and cattle 16
2.5. Cottonseed meal 17
2.5.1 Nutrient characteristics of cottonseed meal 17
2.5.2 Effect of cottonseed meal supplementation on performance of feedlot sheep and cattle 19
2.6. Protein quality and its importance in ruminant nutrition 20
2.6.1 Protein quality of oilseed meals 24
2.7 Bottom line 26
Chapter 3 27
Materials and Methods 27
3.1 Introduction 27
3.2 Experimental diets 27

3.3. Growth (feedlot) trial- Experiment 1 28
3.3.1 Animals and routine procedures 28
3.3.2 Experimental design 30
3.3.3 Adaptation period 31
3.3.4 Feedlot growth period 32
3.3.5 Facility design and management 33
3.3.6 Slaughtering and carcass parameters 33
3.3.7 General processing of samples prior to laboratory analyses 35
3.3.8 Chemical analyses 35
3.3.9 Calculations and parameters measured 36
3.3.10 Statistical analysis 38
3.4 4×4 Latin square design - Experiment 2 39
3.4.1 Experimental design 39
3.4.2 Animals and management 39
3.4.3 Adaptation period and facility design 40
3.4.4 Sample collection period 41
3.4.4.1 Digestibility sampling 41
3.4.4.2 Rumen fermentation parameters 41
3.4.4.3 *In sacco* bag preparation and incubation 42
3.4.5 Chemical analysis 44
3.4.6 Calculations and parameters measured 44
3.4.7 Statistical analysis 46
Chapter 4 47
Results and Discussion - Experimental Diets 47
4.1 Introduction 47
4.2.1 Nutrient composition of experimental diets 47
4.2.2 Acid detergent insoluble nitrogen content and visual appraisal of experimental diets 49
4.2.3 Nutrient characterisation of raw materials 54
Chapter 5 56
Results and Discussion - Feedlot Growth Trial 56
5.1 Introduction 56
5.2.1 The effect of different protein concentrates on the mean growth performance and feed intake of feedlot lambs 56
5.2.2 The effect of different protein sources on carcass characteristics 68
Chapter 6 73
Results and Discussion - 4×4 Latin Square Design 73
6.1. Introduction 73

6.2 The effect of different protein concentrates on the dry matter intake and nutrient digestibility of mature cannulated wethers 73
6.3 The effect of different protein concentrates on the 16h *in situ* ruminal crude protein and dry matter disappearance 77
6.4 The effect of different protein concentrates on rumen fermentation 80
Chapter 7 87
Conclusion 87
Chapter 8 89
Critical Evaluation 89
References 92

Declaration

I, Ashley Grimsell, declare that the thesis/dissertation, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: ……

Date: ……

Acknowledgements

I would like to extend my gratitude to the following individuals for their invaluable contributions, which assisted with the completion of this dissertation; your support is greatly appreciated.

Thank you to my supervisor, Professor Lourens Erasmus, for affording me the opportunity to do this research project. In addition, many thanks for all the support and guidance that he has provided to me throughout my undergraduate and postgraduate studies. I have sincere appreciation for all the time and patience that he has invested in me.

To my friends and family, especially my mother Julia and sister Andrea, thank you for all your help during sampling, as well as your endless motivation and support.

Thank you to Mr Roelf Coertze, who assisted with the statistical analyses of the data.

To Voermol Feeds, thank you for granting me the opportunity to conduct this trial and for the provision of funding for this research project. To Mr Ulrich Müller from Voermol Feeds, thank you for your assistance throughout the course of the trial; it is sincerely appreciated.

Magohu Alfred Phahlamohlaka (Freddy), thank you for the numerous hours that you have invested in assisting me with the trial. In addition, thank you for all the assistance with the sheep, it is sincerely appreciated.

Thank you to all the staff from the Hillcrest Experimental Farm for assisting me.

Thank you to the South African Society for Animal Science (SASAS) for awarding me a postgraduate bursary.

My Lord and Saviour, thank You for blessing me so abundantly;

“Ek weet wat Ek vir julle beplan, sê die Here: voorspoed en nie teenspoed nie; Ek wil vir julle ‘n toekoms gee, ‘n verwagting!” - Jeremia 29:11

Summary

Effect of protein source and quality on feedlot growth performance and rumen fermentation characteristics of Döhne Merino lambs

By

Ashley Grimsell

Supervisor: Professor L.J. Erasmus

Department: Animal and Wildlife Sciences

Faculty: Natural and Agricultural Sciences

University of Pretoria

Pretoria

Degree: MSc(Agric) Animal Science: Animal Nutrition

The importance of protein quality in the formulation of ruminant rations has been regarded as inconsequential in the past. Moreover, the use of agro-industrial by-products as feed ingredients have become attractive alternatives in the animal feed industry, whereby several of these products characteristically have alternative protein qualities. Dried brewer's grains (DBG) is a by-product originating from the beer manufacturing industry, frequently incorporated in the diets of feedlot lambs, however, has caused obstacles in some feed mills in South Africa. The oilseed by-products: canola-, soybean- and cottonseed meal, have all been proposed as suitable protein sources which can be included lamb feedlot diets. In this study, DBG, canola meal, soybean meal and cottonseed meal were incorporated as protein sources into concentrate-rich feedlot lamb diets. All experimental diets were formulated to contain 14% crude protein (CP) and 10.2 MJ ME/kgDM. The four experimental treatments differed in terms of the inclusion of the primary protein source which was either DBG, canola meal, soybean meal or cottonseed meal. Two experiments were conducted. The first experiment, comprising a feedlot growth experiment, utilized a randomised complete block design (RCB) as the experimental design. It was conducted using 200 Döhne-Merino lambs in order to investigate the effect of protein quality on the growth performance, feed intake and carcass parameters. The average total weight gained over the duration of the feedlot growth trial, as well as the average daily gain (ADG) of the canola meal treatment, was lower ($P<0.05$) than the other three treatments. Higher ($P<0.05$) feed intakes were realised on the cottonseed meal diet as opposed to the other three treatments. Additionally, lower ($P<0.05$) feed intakes were realised on the DBG diet when compared to the other three treatments. The feed

conversion ratio (FCR) of the DBG diet was better ($P<0.05$) than the canola-, soybean- and cottonseed meal treatments, respectively. Conversely, the FCR realised for the canola meal diet was poorer ($P<0.05$) than the other three treatments. The lower ($P<0.05$) final live weights of the lambs from the DBG treatment, in comparison to those from the canola meal and cottonseed meal treatments, translated into lower ($P<0.05$) hot and cold carcass mass. Carcass classification remained unaffected ($P>0.05$) by treatment. Nevertheless, dissimilarities ($P<0.10$) were detected in the dressing percentages of the canola meal treatment and cottonseed meal treatment, whereby the dressing percentages of the former were higher. The second experiment entailed four mature cannulated wethers being subjected to a 4×4 Latin square design in order to investigate the effect of protein quality on the intake, apparent total tract digestibility, degradability and some rumen fermentation parameters of the sheep. Feed intake was greater ($P<0.05$) for the DBG diet as opposed to the soybean meal diet. Thus, the feed intake results of the two separate experiments differed from one another. The apparent DM digestibility of the DBG diet was lower ($P<0.10$) than the soybean meal diet. A higher ($P<0.05$) apparent CP digestibility was realised on the soybean meal diet, as opposed to the DBG and canola meal diets. The apparent total tract starch and NDF digestibility remained unaffected ($P>0.05$) by treatment for the current study. A lower dry matter (DM) degradability estimate ($P<0.05$) was realised for DBG, when compared to the other three treatments. No differences ($P>0.05$) were detected in the CP degradability estimates between the four respective protein concentrates, however could have been predisposed to experimental error. Average ruminal pH was higher ($P<0.05$) for the cottonseed meal diet, as opposed to the other three treatments and average ruminal ammonia nitrogen concentration did not differ ($P>0.05$) between treatments. The results advocate that protein quality has the potential to influence growth parameters, digestibility and rumen fermentation in sheep. Further research needs to be conducted on the inclusion levels of the respective protein concentrates and the breakpoint in the feeding period, where protein quality can be regarded as not important anymore.

Abbreviations

º C	-	Degree Celsius
AA(s)	-	Amino acid(s)
ADF	-	Acid detergent fibre
ADG	-	Average daily gain
ADIN	-	Acid detergent insoluble nitrogen
AOAC	-	Assosciation of Official Analytical Chemists
BW	-	Body weight
$BW^{0.75}$	-	Metabolic body weight
Ca	-	Calcium
CALabs	-	Central Analytical Laboratories
Cl	-	Chlorine
CMW	-	Cape Mohair and Wool
CP	-	Crude protein
DBG	-	Dried brewers grain
DM	-	Dry matter
DMI	-	Dry matter intake
DOF	-	Days on feed
EE	-	Ether extract
EPD	-	Effective protein degradability
FCR	-	Feed conversion ratio
Fe	-	Iron
FI	-	Feed intake
FME	-	Fermentable metabolisable energy
g(s)	-	Gram(s)
GE	-	Gross energy
GLM	-	General linear model
H_2SO_4	-	Sulphuric acid
H_3PO_4	-	Phosphoric acid
iDM	-	Initial dry matter
IVOMD	-	*In Vitro* organic matter digestibility

K	-	Potassium
kg	-	Kilogram
LW	-	Live weight
MC	-	Microbial contamination
MCP	-	Microbial protein
ME	-	Metabolisable energy
mg	-	Milligram
MJ	-	Mega joule
ml	-	Millilitre
N	-	Nitrogen
Na	-	Sodium
NDF	-	Neutral detergent fibre
NFC	-	Non-fibre carbohydrates
NH_3	-	Ammonia
NH_3-N	-	Ammonia nitrogen
NE	-	Net energy
NPN	-	Non-protein nitrogen
NRC	-	National Research Council
NSC	-	Non-structural carbohydrates
OM	-	Organic matter
P	-	Phosphorus
ppm	-	Parts per million
RCB	-	Randomised complete block design
RDP	-	Rumen degradable protein
RUP	-	Rumen undegradable protein
S	-	Sulphur
SA	-	South Africa
SAMM	-	South African Mutton Merino
SAS	-	Statistical analysis system
SE	-	Standard error
TEAA	-	Total essential amino acid(s)
TDN	-	Total digestible nutrients

TMR	-	Total mixed ration
UDP	-	Undegraded dietary protein
USA	-	United States of America
VFA(s)	-	Volatile fatty acid(s)
WBG	-	Wet brewers grain
Zn	-	Zinc

Abbreviations of Amino Acids

Ala	-	Alanine
Arg	-	Arginine
Asn	-	Asparagine
Asp	-	Aspartic acid
Gln	-	Glutamine
Glu	-	Glutamic acid
Gly	-	Glycine
Cys	-	Cysteine
His	-	Histidine
Ile	-	Isoleucine
Leu	-	Leucine
Lys	-	Lysine
Met	-	Methionine
Phe	-	Phenylalanine
Pro	-	Proline
Ser	-	Serine
Thr	-	Threonine
Trp	-	Tryptophan
Tyr	-	Tyrosine
Val	-	Valine

List of Tables

Table 2.1	Nutrient composition of dried brewers grain…….....	6
Table 2.2	Amino acid composition of dried brewers grain…….....	7
Table 2.3	Nutrient composition of solvent extracted canola meal…….	10
Table 2.4	Fermentable metabolisable energy, metabolisable energy, effective ruminal degradable protein and digestible undegradable protein values for ruminants……	11
Table 2.5	Amino acid composition of canola meal……..	12
Table 2.6	Nutrient composition of 49% CP solvent extracted soybean meal……	15
Table 2.7	Amino acid composition of soybean meal..……	15
Table 2.8	Nutrient composition of 41% mechanically extracted cottonseed meal……	18
Table 2.9	Amino acid composition of cottonseed meal……..	18
Table 3.1	Ingredient composition of the four experimental diets, each diet containing a different primary protein source……...	28
Table 3.2	Experimental design of the 4×4 Latin square design depicting the allocation of wethers to treatments during different experimental periods.....	39
Table 3.3	Schedule of rumen fluid collections and the sampling day on which it occurred, during each experimental period…….....	42
Table 3.4	The bag numbers and weight of sample placed in each bag, for the *in sacco* procedure…….....	44
Table 4.1	Chemical composition of the experimental diets, mixed as batch 2……	48
Table 4.2	Acid detergent insoluble nitrogen content of the different protein sources for both batches 1 and 2……..……	49
Table 4.3	Nutrient composition of raw materials on a dry matter basis…….....	54
Table 4.4	Chemical composition of the protein concentrates used in the experimental diets, determined by laboratory analyses…….....	55
Table 5.1	The effect of different protein concentrates on mean (±SE) feed intake and growth performance of feedlot lambs……...	58
Table 5.2	The effect of different protein concentrates on mean (±SE) weekly dry matter intake of feedlot lambs…….	64
Table 5.3	The effect of different protein concentrates on mean (±SE) weekly feed conversion ratios (DM basis) of feedlot lambs……	67
Table 5.4	The effect of different protein concentrates on mean (±SE) hot carcass mass, cold carcass mass and dressing percentage of feedlot lambs…….	69
Table 6.1	The effect of protein concentrate on mean (±SE) dry matter intake, dry matter intake per kilogram metabolic body weight and total tract apparent nutrient digestibility in sheep ……...	74
Table 6.2	The effect of protein concentrate on the mean (±SE) ruminal *in situ* dry matter and crude protein disappearance at 16 hours in sheep……	78
Table 6.3	The effect of protein concentrate on mean (±SE) ruminal pH and ruminal ammonia nitrogen concentration in sheep fed four different protein concentrates…….	80

List of Figures

Figure 2.1 Fate of dietary crude protein in the ruminant animal…….……. 23

Figure 3.1 Random assignment of treatments to pens with accompanying average pen weights, post-blocking……... 31

Figure 3.2 Timeline of the feedlot experiment with respective events being outlined…… 33

Figure 3.3 Photographic image of the lamb carcass grading system, using Meat Matrix Grading at the Cavalier abattoir…….. 34

Figure 4.1 Treatment 1 (DBG) from batch 1, in the feed bag prior to feeding…… 51

Figure 4.2 Treatment 2 (canola meal) from batch 1, in the feed bag prior to feeding…… 51

Figure 4.3 Treatment 3 (soybean meal) from batch 1, clumped pellets removed prior to feeding…… 52

Figure 4.4 Treatment 4 (cottonseed meal) from batch 1, in the feed bag prior to feeding…… 52

Figure 4.5 Treatment 1 (DBG), batch 2 in the feed bag prior to feeding……..... 52

Figure 4.6 Treatment 2 (canola meal), batch 2 prior to feeding…… 53

Figure 4.7 Treatment 2, batch 2 undesirable pellets removed prior to feeding…….. 53

Figure 4.8 Treatment 3 (soybean meal), from batch 2 prior to feeding…… 53

Figure 4.9 Treatment 4 (cottonseed meal), from batch 2 prior to feeding……..... 54

Figure 5.1 Weekly mean daily gain of lambs receiving different protein concentrates in the feedlot…….. 61

Figure 5.2 Weekly mean body weight of lambs receiving different protein concentrates in the feedlot…… 62

Figure 5.3 Weekly fluctuation in feed intake (as is) of feedlot lambs receiving different protein concentrates in the feedlot…….. 65

Figure 5.4 Carcass classification of feedlot lambs fed different protein concentrates……... 70

Figure 6.1 Fluctuation in ruminal pH over a 24-hour period in sheep fed different protein concentrates…… 82

Figure 6.2 Photographic image of the cannulated sheep's (P1303) ruminal epithelium …..… 83

Figure 6.3 Magnified image of Figure 6.2, note damage of ruminal papillae……..……... 83

Figure 6.4 Photographic image of a feedlot lambs' ruminal epithelium…… 84

Figure 6.5 Variation in ruminal ammonia-nitrogen concentration over a 24-hour period in sheep fed different protein concentrates…… 86

Chapter 1

Introduction and Motivation

The finishing off of lambs in feedlots are increasing in South Africa (Brand *et al.*, 2018). Feedlot operations are aiming to increase feed efficiency as producers exercise more control over the diet; thus allowing for the formulation of diets which more accurately meet animal requirements, compared to those fed forage-based diets (NRC, 2007). However, due to the small profit margin in feedlot production systems, it is necessary to consider the incorporation of by-products originating from various processing industries into rations. This will ultimately decrease the cost of production and diminish pollution (Nagalakshmi *et al.*, 2003a; Faccenda *et al.*, 2018).

Protein is regarded to be the most costly nutrient of the diet; subsequently, it is more economical to include protein sources into mixed rations (NRC, 2007). Several protein sources originating from agro-industrial processing have frequently been included in ruminant rations, specifically: dried brewer's grains, canola meal, soybean meal and cottonseed meal (Bovolenta *et al.*, 1998; Wiese *et al.*, 2003; Silva *et al.*, 2016). Due to the unique digestive systems of ruminants (Faccenda *et al.*, 2018), they are generally able to utilize these products efficiently.

However, the use of dried brewer's grains (DBG) in ruminant diets appears to be on the decline; this seems to be a global trend (Westendorf &Wohlt, 2002). Several factors have contributed to this shift in feedstuff use; increased competition from the dairy industry for the wet product, elevated costs associated with the production of the dried product, improvements in the management and transportation of the wet variety and lastly, the seasonality of beer production (Westendorf &Wohlt, 2002). Other obstacles associated with the use of dried brewers grain as a feedstuff, have become apparent in the South African industry. Firstly, drying requires electricity, which is an expensive commodity locally. Moreover, feed manufacturing factories have been confronted with challenges such as the spontaneous combustion of the product in the feed production mills, dried brewer's grain having an unpredictable supply and the product being prone to accumulation in the feed mill (2018, U. Müller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380, South Africa).

True protein supplements encompass supplements which originate from animal and plant sources. However, plant-based protein concentrates such as the aforementioned oilseed meals, have received considerable interest in animal nutrition (Freer &Dove, 2002). This

interest will continue to grow as the use of protein supplements of animal origin have notably posed numerous risks. Although resistant to ruminal degradation (NRC, 2007), animal protein supplements have the potential to transfer disease to the human population if fed to animals, in particular meat and bone meal and blood meal (Freer &Dove, 2002).

All of the above-mentioned protein sources have different nutritional qualities; with specific reference to their amino acid profiles and protein degradability characteristics (McDonald *et al.*, 2011; Khalid *et al.*, 2012), which will ultimately determine the extent to which they will affect production. The degree to which an animal will respond to any protein source will depend on its requirement for metabolisable energy (ME) , rumen degradable protein (RDP) and rumen undegradable protein (RUP) and the interaction with other nutrients in the diet (Freer &Dove, 2002). In order to increase the supply of microbial protein (MCP) to the ruminant, RDP supplements are fed, stimulating the growth and multiplication of microbes (McDonald *et al.*, 2011). Conversely, protein concentrates characteristically high in RUP, can additionally be supplied in order to increase the total protein flow to the duodenum (Armentano *et al.*, 1986) whilst simultaneously altering the amino acid profile reaching the small intestine, thus achieving elevated levels of production (Stern *et al.*, 2006; Khalid *et al.*, 2012).

Limited research exists on the effects of providing supplemental protein in the form of RUP on the performance of feedlot lambs (Beauchemin *et al.*, 1995). Additionally, a perception exists that protein quality is not important in the formulation of feedlot diets for ruminants (Vasconcelos &Galyean, 2007). Therefore, further research needs to be conducted.

The objective of the study was to investigate the impact of four different plant based protein sources differing in protein quality on lamb feedlot growth performance and rumen fermentation dynamics.

In the following chapter, the nutrient characteristics of the primary plant proteins generally used in sheep feedlots and their impact on production will be discussed. In Chapter 3, 4, 5 and 6 materials and methods as well as the results and discussion will be covered. Ending off with a short section on general conclusions and a critical review.

Hypotheses

H_0: The inclusion of either dried brewers grain, canola meal, soybean meal or cottonseed meal as primary protein sources that differ in protein quality will not affect the growth performance and rumen fermentation dynamics of feedlot lambs.

H_1: The inclusion of either dried brewers grain, canola meal, soybean meal or cottonseed meal as primary protein sources that differ in protein quality will affect the growth performance and rumen fermentation dynamics of feedlot lambs.

Chapter 2

Literature Review

2.1 Overview of sheep feedlots

Consumers demand for lamb and mutton is on the rise in South Africa (Burger *et al.*, 2013). This requires alternative methods to be employed other than fattening sheep under extensive conditions, which is commonly practised (Mentz *et al.*, 2015; Webb, 2015). A feedlot is classified as an intensive production system which requires a limited land area, making use of concentrate diets in order to round off animals (NRC, 2007). The predominant sheep breeds found in South African small stock production systems originate from the Merino and Döhne-Merino breeds (Nolte &Ferreira, 2004; Cloete *et al.*, 2012). These breeds are therefore common in feedlot operations in South Africa. Lambs which enter a commercial feedlot are fattened under intensive conditions on concentrate diets for a limited period of time in order to utilize the high growth potential of young lambs for optimum production (Beauchemin *et al.*, 1995; Webb &Erasmus, 2013). The growth rate and development of any animal is predetermined by its genotype, but can moreover be influenced by nutritional factors and the environment (Brand *et al.*, 2017). Ruminants are, prior to slaughter, finished off on feedlot diets in order to realise maximal growth, while reducing the total days on feed. This will ultimately lead to reduced production costs and higher carcass yields. The elevated energy content of concentrate rations allow for a higher average daily gains (ADG) to be realised in feedlot lambs, compared to lambs finished off on pasture-based diets (Brand *et al.*, 2017).

Farmers often opt to finishing off their animals in feedlots prior to slaughter when grass or forage availability is low and the quality thereof, poor (Nolte &Ferreira, 2004; Ponnampalam *et al.*, 2005). In addition, livestock theft (Cornelius, 2017), undesirable growth performance exhibited on extensive systems, unstable weather conditions (Webb *et al.*, 2018) and predation (Kerley *et al.*, 2017) are major hurdles facing sheep producers in South Africa. These factors thus motivate producers to finish off lambs under intensive conditions, such as feedlots. This ensures that market demand can be met and in addition, the supply of a more consistent carcass to the consumer, in terms of quality and weight, can be realised (Webb &Erasmus, 2013).

Lambs enter commercial feedlots post-weaning, at an age of approximately 3 months. Animals remain in the feedlot until a specific target body weight is achieved or after a predetermined period of days on feed (DOF) (Sheridan *et al.*, 2003). In the South African feedlot industry, lambs are usually slaughtered after the completion of a predetermined

number of days in the feedlot, as opposed to attaining a specific body weight (Brand *et al.*, 2017).

Pelleted diets have the potential to yield higher levels of animal productivity and reduce the selection ability of feed constituents by sheep. Casey & Webb (1995) demonstrated that wethers fed a pelleted diet had higher average daily gains which caused a significant decrease in the number of days on feed , when compared to wethers fed an unpelleted diet (Casey &Webb, 1995). Feed intake (FI) and feed conversion ratios (FCR), furthermore were improved in the animals fed the pelleted ration. The authors highlighted that sheep are selective feeders, resulting in higher intakes of the pelleted ration, as sheep could not select specific feed components. Pelleting additionally enhances the palatability of feedstuffs, controls the intake ratio of roughage to concentrate of lambs (Stanton *et al.*, 2006) and reduces the dustiness of the feed (NRC, 2007). However, feeding a pelleted diet negatively impacts the economics of a feedlot due to the increased costs of production (Bowen *et al.*, 2006).

The sections that follow, will briefly discuss the nutrient profiles of four different protein feeds that differ in quality and how it impacts the performance characteristics of ruminants in feedlots. These protein concentrates include dried brewers grain, canola meal, soybean meal, and cottonseed meal.

2.2. Dried brewers grain

2.2.1 Nutrient characteristics of dried brewers grain

Brewers grains (brewers spent grains) exist in either one of two forms: dried or wet brewers grain (Westendorf &Wohlt, 2002). Wet brewers grains (WBG) are dried in order to produce the dried product, which can successfully be stored and has an elevated nutrient concentration (Bovolenta *et al.*, 1998). Hence, the dried product is less bulky, allowing for lower transportation costs and demands less storage space. The main animal feed market segments seeking the acquisition of brewer's grains are the dairy cattle and a portion of the beef feedlot industry (Westendorf &Wohlt, 2002).

Westendorf & Wohlt (2002) and Shaver (2013) define dried brewers grain as a by-product of beer production; a residue consisting of barley malt exclusively, or a combination of grain products that have been dried and extracted. The composition of dried brewer's grains is subsequently often variable due to the inclusion of varying proportions of other grain residues originating from maize or rice. Brewers grains can be considered a palatable feedstuff with a medium protein content, but is also a concentrated source of digestible fibre and has an average ME content of 11.2 MJME/kgDM (McDonald *et al.*, 2011).

The two major components of dried brewer's grain, irrespective of region or brewery of production, are crude protein (CP) and neutral detergent fibre (NDF), as shown in Table 2.1. Dried brewer's grain has been described as a valuable source of protein (Westendorf &Wohlt, 2002) as it has the ability to provide a considerable amount of the required supplemental protein and undegraded dietary protein (UDP) (Table 2.4) in the diet of ruminants, as well as being able to supply highly digestible fibre (Westendorf &Wohlt, 2002; McCarthy *et al.*, 2013). The CP content of dried brewer's grain can range between 25-33%, thus rendering it a significant protein source in ruminant nutrition and it can furthermore be considered a medium energy source with ME ranging from 10.5-12MJ/kgDM.

Table 2.1 Nutrient composition of dried brewers grain[1]

Nutrient	% DM basis
DM	92.00
CP	25.00
CF	14.00
ADF	24.00
NDF	49.00
eNDF	18.00
EE	7.50
Ash	4.00
Ca	0.30
P	0.58
K	0.10
Cl	0.15
S	0.32
Zn[2]	78.00

[1]Adapted from Preston (2016)
[2]Zn presented as ppm

Bovolenta *et al*. (1998) describes dried brewer's grain as a protein concentrate with low biological value, owing to its low lysine (Lys) content (Table 2.2), which is an essential amino acid required for growth. The rumen degradability of the protein found in dried brewers grain is lower than that of wet brewer's grain (Armentano *et al.*, 1986; McDonald *et al.*, 2011). This can in turn have a negative impact on rumen microbial growth, which could hinder the digestion of other nutrients in the diet by the ruminant (Faccenda *et al.*, 2018). In addition, the utilisation of the protein fraction of DBG is subsequently lower than that of WBG; authors attribute this to the heat that the brewers grain is exposed to during processing (Rogers *et al.*, 1986). The NRC (2007) have also authenticated that an alteration in the nutritional quality of DBG may result due to drying. When ruminants are fed diets containing a combination of DBG and non-protein nitrogen sources (NPN) such as urea, DBG has the potential to supply all the indispensable amino acids (AA) (McCarthy *et al.*, 2013). The protein quality of DBG is

regarded lower than that of other protein feedstuffs such as soybean meal (Westendorf &Wohlt, 2002).

Dried brewers grain furthermore contains a considerable amount (approximately 11% of the grain DM) of non-structural carbohydrates (NSC) and fat (>5% of the grain DM), thus could also be considered as a potential source of energy in ruminant diets (Westendorf &Wohlt, 2002).

Neutral detergent fibre constitutes a great portion of the DM of the dried brewers grain (Table 2.1) and it has been reported that the digestibility of the hemicellulose and NDF fractions are relatively high (Bovolenta *et al.*, 1998). Due to brewer's grain formerly being fermented in the beer production process, the majority of the starches and sugars have been removed; subsequently, energy losses in the form of methane are notably lower than other concentrate feeds which are high in starch (McDonald *et al.*, 2011). According to McDonald *et al*. (2011), the mineral contents of brewers grains are generally low, with specific reference to sodium (Na), calcium (Ca) and potassium (K) (Westendorf &Wohlt, 2002), however the phosphorous (P) content is high (Table 2.1). Dried brewers grain is therefore regarded as a pivotal ingredient in the formulation of ruminant rations due to its unique nutrient composition and low cost (Westendorf &Wohlt, 2002).

Table 2.2 Amino acid composition of dried brewers grain[1]

Amino Acid	% CP (DM basis)
Arg	5.77
His	2.00
Ile	3.85
Leu	7.85
Lys	4.08
Met	1.70
Cys	1.85
Phe	4.60
Thr	3.58
Trp	0.98
Val	4.75
TEAA	39.16

[1]Adapted from Westendorf & Wohlt (2002)

2.2.2 Effect of dried brewers grain supplementation on performance of feedlot sheep and cattle

The elevated fibre content of dried brewers grain makes it a popular feed for ruminants (Bovolenta *et al.*, 1998), however high inclusion rates could lead to reduced performance

(Westendorf &Wohlt, 2002). On the contrary, Bovolenta *et al.* (1998) suggested that higher growth rates are potentially attainable in lambs owing to the low rumen degradability of the protein in DBG (Armentano *et al*., 1986).

Lower ADG (1113g/day) values were reported, however not significant, in young growing bulls fed diets containing higher (36%) inclusion rates of DBG when compared to the soybean meal only- and lower (17%) DBG diets, in a study conducted by Öster *et al.* (1977). In the study conducted by Bovolenta *et al*. (1998), it was found that as the DBG content of the diets increased, the dry matter intake (DMI) was significantly reduced. The authors attributed the lower feed intakes to the reduced palatability of the DBG, also supported by Westendorf & Wohlt (2002). Similarly, growing cattle fed diets containing greater levels (36%) of DBG, had lower feed intakes (0.2kg lower) during the first two months of the trial (Öster *et al.*, 1977), compared to those fed soybean meal or DBG diets with a lower (17%) DBG inclusion level. Lambs did, however, exhibit higher (more efficient) feed conversion efficiencies when Lucerne hay was replaced with incrementally higher levels of DBG (Bovolenta *et al.*, 1998). On the contrary, Öster *et al.* (1977) reported that a reduced FCR in young bulls fed high DBG diets occurred, attributing this to lower energy intakes due to the fibrous nature of DBG.

Results pertaining to the influences of DBG on carcass characteristics are contradictory. Bovolenta *et al.* (1998) reported that lambs fed diets containing high levels of DBG produced fatter carcasses, with the authors attributing this result to the oversupply of protein. On the contrary, Öster *et al.* (1977) reported leaner carcasses in young bulls fed a diet containing higher levels of DBG, when compared to animals fed soybean meal diets or diets with lower DBG inclusion levels.

Feeding DBG in feedlots has displayed potential health benefits to ruminants; by replacing maize or other feed ingredients with DBG, improved growth rates of steers were observed and subsequently, the incidence of liver abscesses and ruminal keratosis were reduced (Öster *et al.*, 1977; Westendorf &Wohlt, 2002).

In addition, DBG has the ability to influence the digestibility of nutrients. This was demonstrated by Bovolenta *et al.* (1998), who reported improvements in all the apparent digestibility coefficients measured. Similarly, elevated levels of DM digestion in the rumen can be expected when feeding DBG, as opposed to WBG, due to reduced rates of passage of the dry product (Westendorf &Wohlt, 2002).

2.3. Canola meal

2.3.1 Nutrient characteristics of canola meal

Canola meal is the North-American trademark name for rapeseed meal (McDonald *et al.*, 2011) and is the term which will be used throughout the study, in order to avoid confusion. Canola production has increased by three-fold over the past few years in South Africa (Agenbag, 2015). Canola meal is obtained after the oil has been extracted using a prepress or direct solvent-extraction procedure (He *et al.*, 2013; Shaver, 2013). For a considerable number of years, canola meal has been underestimated in terms of its value as a useful protein source for ruminant livestock. This was mainly due to the fact that the mineral and protein content of the whole canola seed is only 50% of the value in canola meal (He *et al.*, 2013). Second to soybean meal, canola meal is one of the most universally used protein sources for livestock feeds (Canola Council of Canada, 2015).

The variation in the nutrient composition (Table 2.3) of canola meal can be associated to several factors, namely: the cultivar, the environmental conditions that the crop experienced during growth, the harvesting conditions and finally, the processing of the meal and seed (Canola Council of Canada, 2015). Canola meal has a higher fibre, lower protein and lower gross energy value when compared to de-hulled soybean meal. The fibre content of canola meal is triple that of which is present in soybean meal, which resultantly dilutes the energy value of the canola meal (Table 2.4). This is primarily due to the poor digestibility of the fibre (Dale, 1996; Lardy &Anderson, 2002). De-hulling processes can, however, be employed in order to minimise the negative effects that the fibre fraction has on the energy content of canola meal (Lardy &Anderson, 2002). Canola meal has higher concentrations of essential minerals and B-vitamins when compared to soybean meal (Bell, 1993).

Table 2.3 Nutrient composition of solvent extracted canola meal[1]

Nutrient	% DM basis
DM	91.00
CP	40.00
CF	12.00
ADF	19.00
NDF	27.00
eNDF	23.00
EE	2.70
Ash	8.00
Ca	0.70
P	1.12
K	1.20
Cl	0.07
S	0.72
Zn[2]	63.00

[1]Adapted from Preston (2016)
[2]Zn presented as ppm

The CP content of canola meal is however variable. He *et al*. (2013) reported a value of 35%, Lardy & Anderson (2002) published a value of 40% and the Canola Council of Canada (2015) reported a value of between 36-39 %. Canola meal can be considered an excellent protein supplement for small ruminants, such as sheep, due to it being a useful source of RDP (Zagorakis *et al.*, 2015; Nair *et al.*, 2016), as demonstrated in Table 2.4. When compared to soybean meal protein, the degradation of canola meal protein is more rapid in the rumen (Bell, 1993). Previous studies have reported that the effective degradability of the protein found in canola meal tends to vary between 44.3% - 74% (Zagorakis *et al.*, 2015). Canola meal is thus commonly included in rations as a protein concentrate, contributing to the ruminal ammonia and amino acid pools, which become available to rumen microbes (Ponnampalam *et al.*, 2005). This subsequently improves the rumen fermentation of the NDF, as well as acid detergent fibre (ADF) fractions of the diet; this enables cellulolytic bacteria to efficiently proliferate under these conditions (Nair *et al.*, 2016). However, it needs to be considered that a large quantity of dietary protein originating from canola meal can potentially be lost in the ruminant due to the high rumen degradability of the protein (Bell, 1993). Conversion of the protein in the rumen to ammonia by rumen microbes takes place, which is then absorbed across the rumen wall and subsequently used in the liver to produce urea. This is then excreted, which is considered a waste of protein if there is not sufficient fermentable metabolisable energy (FME) available (Bell, 1993; Freer &Dove, 2002).

Table 2.4 Fermentable metabolisable energy, metabolisable energy, effective ruminal degradable protein and digestible undegradable protein values for ruminants[1]

	DBG	Canola meal	Soybean meal	Cottonseed meal
DM (g/kg DM)	860	890	905	924
ME (MJ/kgDM)	12.2	12.0	13.3	11.1
FME (MJ/kgDM)	9.9	11.0	12.7	8.8
EE (g/kg DM)	67	28	17	66
CP (g/kg DM)	249	400	497	375
ERDP @k=0.05(g/kg DM)	91	288	313	222
DUP @k=0.05(g/kg DM)	132	57	146	109
ERDP (as a % of CP)[2]	36.6	72.0	63.0	59.2
DUP (as a % of CP)[2]	53.0	14.3	29.4	29.1

[1]Adapted from AFRC (1993)

[2]Calculated

Abbreviations: ERDP- Effective rumen degradable dietary protein; DUP-Digestible undegraded protein; k- rumen digesta outflow rate

The frequent use of canola meal in ruminant livestock rations occurs due to its palatability and amino acid profile (Table 2.5). It supplies adequate levels of methionine (Met); one of the first limiting amino acid of ruminants during production (Canola Council of Canada, 2015), specifically at elevated levels of carcass protein deposition (NRC, 2007). The lysine content of canola meal is conversely lower than that found in soybean meal (Ramachandran *et al.*, 2007; McDonald *et al.*, 2011). Nonetheless, is still beneficial as improved rates of growth can be achieved in ruminants (Khalid *et al.*, 2012). Moreover, canola meal contains high levels of sulphur-containing amino acids, such as cysteine (Cys) (NRC, 2007), which could effectively meet the requirements of wool growth (Canola Council of Canada, 2015) and aid with the synthesis of essential amino acids by microbes (Khalid *et al.*, 2012). The AA profile supplied for absorption by the RUP fraction of canola meal, is consequently better suited to supplying maintenance requirements in ruminants than alternative plant protein sources, according to the Canola Council of Canada (2015).

Table 2.5 Amino acid composition of canola meal[1]

Amino Acid	% CP (DM basis)
Ala	4.36
Arg	6.62
Asp + Asn	7.25
Cys	2.29
Glu + Gln	18.14
Gly	4.92
His	3.39
Ile	3.47
Leu	6.19
Lys	5.92
Met	1.94
Met + Cys	4.25
Phe	4.06
Pro	5.97
Ser	4.00
Thr	4.27
Trp	1.33
Tyr	2.50
Val	4.97

[1]Adapted from the Canola Council of Canada (2015)

The residual oil in the canola meal may result in the improvement of the fatty acid profile, subsequently influencing the fat content in meat producing animals (Canola Council of Canada, 2015). The phosphorus and selenium content of canola meal is high, relative to other oilcake meals (Canola Council of Canada, 2015). In addition, the calcium to phosphorus ratio is also sufficient (McDonald *et al.*, 2011). The phosphorus present in canola meal is valuable to ruminants as it is present in the phytate phosphorus form, allowing for the effective degradation thereof by bacterial phytases in the rumen (Canola Council of Canada, 2015). The temperatures employed during canola meal processing are consequently imperative in ensuring the availability of dietary phosphorus. Elevated processing temperatures lead to reduced phytate degradation in the rumen, resulting in lower dietary phosphorus availability, especially in sheep (Canola Council of Canada, 2015).

Canola meal has become a lucrative feedstuff included in livestock rations over time, although it has some anti-nutritional components that require consideration. Improvements in canola cultivars have reduced the glucosinolate content of the canola meal, which could be harmful to livestock (Dale, 1996). Even when considering the improvements made to the former cultivars, canola meal still needs to undergo rigorous processing. This usually includes employing minimum temperatures, in order to deactivate the myrosinase enzyme, which is potentially harmful to the digestive tract of livestock. The processing can, however, have a

negative impact on amino acid digestibility, specifically that of lysine (Canola Council of Canada, 2015). Maillard reactions take place when feedstuffs are exposure to high temperatures, resulting in the denaturing of proteins and ultimately affecting the availability of nutrients to the animal (Bell, 1993). Tannins present in canola meal, in addition, have the potential to unfavourably effect on the digestibility and availability of protein. Tannins tend to form complexes with proteins, making them unavailable for absorption by ruminants (McDonald *et al.*, 2011).

2.3.2 Effect of canola meal supplementation on performance of feedlot sheep and cattle

Canola meal is becoming an important component of beef cattle finisher rations. Some studies have shown notable improvements in weight gain and feed intake in backgrounded steers that have received canola meal supplementation (Canola Council of Canada, 2015). In the study conducted by Nair *et al*. (2015) canola meal supplementation (from two different cultivars) in backgrounding diets fed to steers led to increased DMI (8.9 vs. 8.3 kg/day) and higher ADG (1.60 vs 1.36 kg/day) values, when compared to the control diet. Distinguishing characteristics of canola meal supplemented diets, irrespective of cultivar, were elevated levels of P, ADF and NDF.

Significant weight gains and improved feed efficiency took place in meat-producing small stock fed canola meal as a part of their ration. In a study conducted in Australia, lambs which were fed a diet containing canola meal showed higher live weight gains (0.272kg/lamb/day) and elevated feed intakes (1.66kg/as is per day) over a 7-week experimental period (Wiese *et al.*, 2003), compared to those receiving lupin- and urea based diets. Furthermore, in a study conducted by Ponnampalam *et al.* (2005), sheep fed diets containing canola meal exhibited lower carcass fat deposition, which authors speculated may have been as a result of the elevated ME content as well as the CP: ME ratio. The higher quantity of vitamins, minerals and sulphur containing amino acids (methionine and cysteine), may subsequently lead to higher levels of production and performance of feedlot lambs, which are fed canola meal (Khan *et al.*, 1997).

Canola meal supplementation can affect the digestibility of some dietary components. Lardy & Anderson (2002), who supplemented finishing beef cattle rations with canola meal, confirmed this. The apparent ADF digestibility of the diet was reduced, yet the CP and energy digestibility were improved in the cattle (Lardy &Anderson, 2002). Similarly, feeding canola meal to heifers also resulted in higher total tract CP digestibility (Nair *et al.*, 2016); authors attributed these improvements to the higher RDP fraction present in the meal, as well as its higher protein digestibility characteristics. Zagorakis *et al.* (2015) additionally demonstrated

that canola meal had nutrient digestibility coefficients that were comparable to that of soybean meal.

2.4 Soybean meal

2.4.1 Nutrient characteristics of soybean meal

Soybean meal is typically produced after de-hulled or whole soybeans have undergone solvent extraction in order to obtain the oil; residual flakes are subsequently finely ground to acquire the meal product (McDonald *et al.*, 2011; Shaver, 2013). Alternatively, soybean meal can also be produced when either soybean chips or oilcake are finely ground after mechanical extraction has been completed (Shaver, 2013). Soybean meal is frequently included in the diets of ruminants as a protein supplement and is regarded as an exceptional protein source. However, due to its popularity as an animal feedstuff, its price has markedly increased (Wanapat *et al.*, 2012), resultantly influencing its availability (Zagorakis *et al.*, 2015). In South Africa, the estimated price for soybean meal for July 2019 was R5 530 per tonne (SAPA, 2018), demonstrating that it is an expensive raw material to include in diet formulation.

The crude protein content, as well as the rumen degradability between soybean meals, vary greatly (Shaver, 2013). This is dependent on the cultivar (Dale, 1996), processing method used and quantity of remaining hull (Mukherjee *et al.*, 2016). Soybean meal is a popular feedstuff in ruminant diets due to the high proportion of RDP (Table 2.4), as well as the high lysine content which it possesses (Santos *et al.*, 1998; Shaver, 2013). The lysine in soybean meal, however, can potentially become unavailable in the event that the meal is overheated (Dale, 1996).

Silva *et al*. (2016) reported that soybean meal has a lower lignin concentration (0.30%), when compared to cottonseed meal (10.60%). From Table 2.6, it is evident that the NDF and ADF content of soybean meal is lower than that of canola meal (Good, 2018). Accordingly, Lardy & Anderson (2002) stated that the digestible energy content of soybean meal is higher than that of canola meal due to the lower hull content in soybean meal. Soybean meal can be a source of some vitamins; McDonald *et al*. (2011) reported that the biotin found in soybean meal is entirely available to the animal and additionally, the NRC (2007) reported a notable Vitamin E content (3.0 parts per million) for soybean meal. Furthermore, the calcium and phosphorus content of soybean meal is higher than that of other cereal grains (McDonald *et al.*, 2011).

Table 2.6 Nutrient composition of 49% CP solvent extracted soybean meal[1]

Nutrient	% DM basis
DM	89.00
CP	54.00
CF	4.00
ADF	6.00
NDF	10.00
eNDF	23.00
EE	1.10
Ash	7.00
Ca	0.35
P	0.75
K	2.30
Cl	0.08
S	0.47
Zn[2]	61.00

[1]Adapted from Preston (2016)
[2]Zn presented as ppm

The protein originating from soybean meal contains all of the indispensable amino acids, however the methionine and cysteine content is limited (Mukherjee *et al.*, 2016), as illustrated in Table 2.7 . Nonetheless, soybean meal supplies sufficient quantities of threonine (Thr), tryptophan (Trp) and as mentioned before, lysine (Ramachandran *et al.*, 2007). Santos *et al.* (1998) have thus described soybean meal as having a reasonably fair AA profile. As a result of its protein characteristics, soybean meal can contribute significantly to the ammonia peptide and amino acids pools, available to rumen microorganisms (Ponnampalam *et al.*, 2005).

Table 2.7 Amino acid composition of soybean meal[1]

Amino Acid	% CP (DM basis)
Arg	7.40
Cys	1.60
Gly	4.50
His	2.40
Ile	4.60
Leu	7.80
Lys	6.10
Met	1.40
Phe	5.50
Thr	3.80
Trp	1.30
Tyr	3.50
Val	5.20

[1]Adapted from Ramachandran *et al*. (2007)

Soybean meal contains several anti-nutritional factors, which predominantly affect non-ruminants. However, phytoestrogens have been shown to affect reproduction in ruminants (Mukherjee *et al.*, 2016).

2.4.2 Effect of soybean meal supplementation on performance of feedlot sheep and cattle

Soybean meal is the most frequently used protein concentrate internationally, followed by canola meal (McDonald *et al.*, 2011). In the study conducted by Ponnampalam *et al.* (2005), higher DMI were realised in lambs fed hay diets which were supplemented with soybean meal (1.05kg) when compared to those receiving the basal diet (0.92kg) (Ponnampalam *et al.*, 2005). In the same study, soybean meal supplementation resulted in reduced carcass fatness in lambs, which is indicative of more lean deposition in these animals. The authors argue that this is presumably due to the increased intake of metabolisable energy and CP: ME ratio.

Stanton *et al.* (2006) reported performance data of feedlot lambs fed different protein concentrates. The lambs fed the ration containing soybean meal exhibited the best feed efficiency values of 4.76 (when compared to other diets) as well as the best increased average daily gains of 0.290kg/lamb/day (Stanton *et al.*, 2006).

In a study conducted by Khan *et al*. (1997), lambs fed diets containing soybean meal (56.00kg) had higher final bodyweights than lambs fed cottonseed meal (52.33kg) or canola meal diets (53.55kg). Daily average body weight gains were notably higher in lambs fed diets containing soybean meal (244g/day) and canola meal (233g/day) than those receiving diets containing cottonseed meal (213g/day) (Khan *et al.*, 1997). The feed intake of lambs receiving the three different oilcake meal diets (i.e. soybean-, canola- and cottonseed meal), however did not differ (Khan *et al.*, 1997). Better feed efficiency values were realised in lambs consuming the canola (8.58) and soybean meal (8.98) diets when compared to those fed the cottonseed meal (9.79) diets respectively (Khan *et al.*, 1997).

In a study conducted with sheep, diets containing soybean meal (control) were compared to two cottonseed meal diets (cottonseed meal only and cottonseed meal with iron (Fe) supplementation) (Ward *et al.*, 2008) . Sheep fed the soybean meal diet yielded higher ADG values (200g/day vs. 170g/day) and better FCR values (5.00 vs 6.47) compared to those fed of the diet containing cottonseed meal exclusively. The authors attributed these findings to the higher nutrient digestibility of the soybean meal diet. However, lambs consuming the soybean meal diets (1.00kg/day) had lower feed intakes than those consuming the cottonseed meal diets (1.10kg/day).

2.5. Cottonseed meal

2.5.1 Nutrient characteristics of cottonseed meal

Cottonseed meal is a by-product produced from the cotton seed oil and cotton fibre industries (Kandylis *et al.*, 1999). This oilcake meal is frequently included in ruminant diets, due to it being an economical protein concentrate (Kandylis *et al.*, 1999). However, its effect on the performance and production of growing ruminants has been inadequately studied (Silva *et al.*, 2016).

The high crude protein content of cottonseed meal, as seen in Table 2.8, makes it a preferred feed ingredient in diet formulation (Kandylis *et al.*, 1999). Nonetheless, the protein content of cottonseed meal has reportedly been variable, ranging from 20-46% (Silva *et al*. 2016). Several authors (Nagalakshmi *et al.*, 2003a; Stern *et al.*, 2006; Wanapat *et al.*, 2012) have described the protein of cottonseed meal to be resistant to protein degradation. Nagalakshmi *et al.* (2003a) reported a range of RUP values from 45-61% which categorises it as a good source of RUP. Due to the high RUP content of cottonseed meal, its inclusion in ruminant diets might have the potential to result in improvements in animal performance (Wanapat *et al.* ,2012).

Cottonseed meal is a valuable source of magnesium, yet a poor source of calcium (McDonald *et al.*, 2011). The shortage of calcium leads to a poor calcium to phosphorus ratio (1:6) in the cottonseed meal and, as a result, deficiencies may develop. The thiamine content of cottonseed meal is adequate, however can be variable. Carotene is an important vitamin which is noted to be lacking in cottonseed meal (McDonald *et al.*, 2011).

Cottonseed meal is a rich source of NDF (13.3 - 32.5%) and can have a high ether extract (EE) value (5.4-14.1%), thus making it suitable for use in the formulation of ruminant rations (Silva *et al.*, 2016). The cottonseed hulls, generally included in the meal product, are thick and high in fibre. These characteristics may lead to the resultant cottonseed meal having a reduced digestibility value and consequently, lower nutritive value (McDonald *et al.*, 2011). Decortication is a process which could alternatively be employed in order to remove all or some of the hulls, thus improving the nutritive value of the cottonseed meal. Silva *et al.* (2016) additionally reported a reduction in the dry matter digestibility of diets that contained higher levels of cottonseed meal, compared to those that contained higher levels of soybean meal. The authors attributed this lower digestibility to the higher lignin content (10.6%) present in cottonseed meal, when compared to soybean meal (0.30%).

Table 2.8 Nutrient composition of 41% mechanically extracted cottonseed meal[1]

Nutrient	% DM basis
DM	92.00
CP	46.00
CF	13.00
ADF	19.00
NDF	31.00
eNDF	23.00
EE	5.00
Ash	7.00
Ca	0.21
P	1.18
K	1.60
Cl	0.05
S	0.39
Zn[2]	64.00

[1]Adapted from Preston (2016)
[2]Zn presented as ppm

Kandylis *et al.* (1999) have characterised cottonseed meal as a protein concentrate that is comparable to soybean meal. The protein originating from cottonseed meal has a good quality, however the lysine, threonine, cysteine, tryptophan and methionine content of this protein is reduced, as demonstrated in Table 2.9 (Ramachandran *et al.*, 2007).

Table 2.9 Amino acid composition of cottonseed meal[1]

Amino Acid	% CP (DM basis)
Arg	11.10
Cys	1.50
Gly	4.50
His	2.60
Ile	3.20
Leu	5.90
Lys	4.10
Met	1.30
Phe	5.40
Thr	3.20
Trp	1.10
Tyr	2.70
Val	4.50

[1]Adapted from Ramachandran *et al.* (2007)

Gossypol, a polyphenolic compound found in cottonseed meal, has limited incorporation in the diets of young animals as it can have adverse effects on their performance. Gossypol exists in either the bound or free form (Ward *et al.*, 2008). Gossypol generally exists in the free form in the whole cottonseed, however becomes bound during the processing and

production of the meal (because of the heat, moisture and pressure). The bound form, poses a slight risk, whereas the free form is toxic. Some researchers have suggested that the bound form is converted to the free form during digestive processes, making it available to the animal (Ward *et al.*, 2008).

Body immune response suppression and histopathological lesions are negative consequences observed when high quantities (40%) of raw cottonseed meal were fed to growing lambs (Nagalakshmi *et al.*, 2003a). The authors speculated that these negative consequences were as a result of all of the free gossypol not being bound in the rumen and subsequently escaping. Nonetheless, McDonald *et al.* (2011) advocates that cottonseed meal is regarded a suitable protein source for mature ruminant animals, as they do not exhibit the negative effects of gossypol toxicity when consuming large volumes of this specific product. This, owing to the fact that mature ruminants are able to detoxify the gossypol by either binding it to soluble proteins in the rumen or by dilution, consequently reducing the absorption thereof (Kandylis *et al.*, 1999). The resulting gossypol-protein complex subsequently has a reduced toxicity when cottonseed meal is fed to ruminants.

2.5.2 Effect of cottonseed meal supplementation on performance of feedlot sheep and cattle

Limited data is available regarding the utilisation and effects of cottonseed meal on the growth and performance of feedlot lambs (Kandylis *et al.*, 1999). However, Stanton *et al.* (2006) reported that lambs fed a ration containing cottonseed meal showed the highest feed intakes of the treatment diets with accompanying increases in average daily gains (281g/lamb/day).

Kandylis *et al*. (1999) conducted a lamb feedlot study using different levels of cottonseed meal and reported that adequate body weight gains, feed intake and improved clinical health were observed. They stated that diets fed to fattening sheep could contain up to 20% cottonseed meal, however careful attention should be given when cottonseed products are fed to ruminants, so as to prevent the over consumption of gossypol, which could lead to gossypol toxicosis (Kandylis *et al.*, 1999). Correspondingly, Nagalakshmi *et al.* (2003a) reported increased average daily gains (87.83 vs. 57.72 g/lamb/day) and feed intakes (601.6 vs. 449.7g/day) in lambs fed diets containing cooked cottonseed meal when compared to the control diet. The authors attributed the body weight gains to the reduced rumen degradability of the protein in cottonseed meal, and the elevated feed intakes due to the palatability of the cottonseed meal. However, rapid body weight gains were reported in previous works when

lambs were fed diets containing a combination of soybean meal and cottonseed meal, when compared to lambs fed soybean meal exclusively (Nagalakshmi *et al.*, 2003a). Feed efficiency depression was not observed when lambs were fed diets containing cottonseed meal (Kandylis *et al.*, 1999). In addition, increasing the cottonseed meal content of the diet did not affect the final body weight, total weight gain or average daily weight gain of lambs receiving the experimental diets as declared by Silva *et al*. (2016).

Beef steers fed high cottonseed meal (HCM) diets, exhibited lower feed intakes (2.7kg/head/day) than those fed low cottonseed meal (LCM) diets (2.8kg/head/day) (Wanapat *et al*., 2012). Another study demonstrated that, as the cottonseed meal content of feedlot steer diets increased, the ADG, feed efficiency and dietary net energy (NE) values decreased (Zinn *et al.*, 1997) .

Several researchers have noted that the free gossypol content of experimental diets were reduced when the diets had undergone pelleting (Kandylis *et al.*, 1999; Nagalakshmi *et al.*, 2003a). Similarly, McDonald *et al.* (2011) specified that the temperatures employed during the expeller process used during oil extraction, adequately inactivates gossypol. Hence, the processing of cottonseed meal diets plays a major role in preventing toxicity and maintaining health status.

Increasing the cottonseed meal content of a diet may reduce the digestibility of the diet. A study conducted by Silva *et al.* (2016) revealed that an increased rate of substitution of soybean meal with cottonseed meal resulted in the reduction of the NDF and DM digestibility of the experimental diets. Ward *et al.* (2008) confirmed similar reductions in DM and organic matter (OM) digestibilities of diets fed to sheep containing cottonseed meal. It can be speculated that the CP digestibility of a diet can be depressed in the event that gossypol toxicity takes place or when the gossypol has affected enzyme functioning (Ward *et al.*, 2008). Conversely, Wanapat *et al*. (2012) stated that previous research has reported elevated nitrogen retention and NDF digestibility in lambs fed cottonseed meal.

It is evident that the effects of cottonseed meal on the performance of ruminants have yielded variable results and subsequently require further research.

2.6. Protein quality and its importance in ruminant nutrition

Protein quality is governed by the proportion and composition of AA, influenced by microbial protein and supplemental protein, which are available for absorption in the small intestine of the animal (Merchen &Titgemeyer, 1992). However, the perception still exists that protein quality is of no value when formulating diets for ruminant animals; in a survey

conducted in the United States of America, several beef feedlot nutritionists failed to take into account RDP requirements when formulating diets (Vasconcelos &Galyean, 2007), yet Erickson *et al*. (2016) regarded this aspect of formulation as important.

The value of a protein source in ruminant ration formulation cannot exclusively be characterised by its CP value, as was previously practised. This is due to the fact that the CP value expressed for ruminants, includes non-protein nitrogen (NPN) compounds (McDonald *et al.*, 2011), which are not necessarily of protein origin. Non-protein nitrogen compounds are classified as small compounds which constitute the following: free AA, amines and amides, peptides, nucleic acids, ammonia and nitrate (Schwab *et al.*, 2003). In addition to the NPN compounds, the contribution of a specific protein source to the RUP and RDP pools, the degree to which it is degraded in the rumen as well as its outflow rate into the small intestine, has been regarded pertinent by nutritionists (Schwab *et al.*, 2003). Alternatively, protein quality has not been considered in formulations for ruminants; the CP content of feedstuffs are relatively easily determined, whereas verifying the contributions of RUP and RDP are complex and the outcomes are frequently variable (Coleman &Moore, 2003). The existence of these two protein pools and their significance in ruminant diets have generated interest, and subsequently encouraged further research regarding the protein degradability of feedstuffs in the rumen.

Emphasis is additionally being placed on the degradability of amino acids in the rumen, which is considered to be a crucial variable in modern protein evaluation systems for ruminant animals (Weisbjerg *et al.*, 1996; Stern *et al.*, 2006). Ruminants have inherent requirements for amino acids that can be satisfied by two fractions. These fractions include rumen degradable protein and rumen undegradable protein. Rumen degradable protein is a protein that becomes available for degradation by microbes in the rumen, whereas RUP enters the small intestine of the ruminant and is ultimately unaffected by microbial degradation (Ørskov &McDonald, 1979; Erasmus *et al.*, 1988; Freer &Dove, 2002). Rumen undegradable protein, also referred to as “bypass protein”, “escape protein” or “undegraded dietary protein” (Freer &Dove, 2002) is crucial in ruminant diets as it is a valuable source of AA, critical for animal survival, growth, maintenance, reproduction and production (Busanello *et al.*, 2017). The AA profile of MCP influences the quality of AA flowing into the small intestines of growing ruminants (Merchen &Titgemeyer, 1992), which in turn influences its efficiency of use within the animal (Boisen *et al.*, 2000). Nevertheless, Merchen & Titgemeyer (1992) reported that in general, ruminants are not considered to have requirements for indispensable AA. This owes to the fact that ruminal microbes are able to synthesise the majority (if not all of) the amino acids required by the host, however the quantities produced are inadequate during elevated levels of production (McDonald *et al.*, 2011). This has since been proven untrue; when the AA supply and

composition thereof was evaluated in studies for growing ruminants, insufficient levels of methionine followed by lysine restricted nitrogen (N) retention in growing lambs (Merchen &Titgemeyer, 1992). The authors have stated that in the case of insufficient MCP production in the rumen, or when the AA requirement by the animal is elevated, AAs from a non-microbial source needs to be supplemented. Correspondingly, the NRC (2007) recommends the consideration of histidine, as it has the potential to be a limiting amino acid during elevated levels of carcass protein production.

It is known that a ruminants primary source of dietary protein is MCP (Freer &Dove, 2002; Stern *et al.*, 2006). A healthy metabolism in the ruminant necessitates a diet which contains adequate levels of essential nutrients coupled with maximal MCP production within the rumen (Busanello *et al.*, 2017). The rumen microbial population produces propionate, butyrate and acetate from dietary carbohydrates in order to supply the majority of the host animals energy requirements (McDonald *et al.*, 2011). In the extensive degradation processes employed by microbes, capitalisation of the energy potential of food occurs, resulting in the synthesis of large quantities of microbial protein. This in turn aids microbial growth and multiplication, given that the concentrations of AA in the rumen are adequate (Broderick &Kang, 1980). The nitrogen component of MCP is acquired from the breakdown of the nitrogen fraction in food, which exists in the form of peptides, amino acids and ammonia (McDonald *et al.*, 2011).

The ruminal and intestinal degradability of dietary proteins differ among feedstuffs, ultimately influencing the amino acids available in the small intestine (Boisen *et al.*, 2000). The degradability of protein in the rumen is determined by several aspects, namely; the physiochemical nature of the protein, the surface area made available for microbial degradation, microbial activity in the rumen, ruminal pH, the passage rate of the protein in the rumen and the protective response exhibited by other elements (Schwab *et al.*, 2003; McDonald *et al.*, 2011). Hence, the influx of undegraded protein into the small intestine is predisposed by the rate and degree to which the nitrogen fraction is degraded in the rumen, the efficiency of synthesising MCP from its precursors, the biological value, as well as the subsequent digestibility of the MCP (McDonald *et al.*, 2011). The origin of MCP, in addition, influences its digestibility. Protein from protozoal origin exhibit a higher digestibility value (ca. 0.90) than those from bacterial origin (ca. 0.75); however, recognition needs to be given to the point that larger quantities of bacterial protein are typically produced in the rumen (McDonald *et al.*, 2011).

The composition of the AA supplied to the small intestine for absorption does not resemble the composition of the AA in consumed feed, which further contributes to the

complex nature of ruminant ration formulation (Merchen &Titgemeyer, 1992). This is as a result of the free AAs rapidly being degraded in the rumen by microorganisms, which are subsequently transformed in these microbial processes (Good, 2018). The outcome thereof is the production of microbial protein with a high biological value (Boisen *et al.*, 2000; Freer &Dove, 2002) and indispensable amino acid content which is assumed to be relatively constant (NRC, 2007; McDonald *et al.*, 2011). The amino acid mixture that is subsequently made available for protein synthesis at tissue level, is determined by the synthetic and degradative processes regularly occurring in the rumen (McDonald *et al.*, 2011). As a result, the diet must satisfy the demands for degradable protein requirements of ruminal microorganisms.

Feed protein supplied to ruminants undergo evaluation in current systems by means of: determination of MCP synthesis, determination of protein degradability in the rumen, digestion of microbial protein as well as feed protein, in the lower gastro-intestinal tract and how effectively amino acids which have been absorbed are utilized (McDonald *et al.*, 2011). Figure 2.1, illustrates the fate of dietary CP in the ruminant animal.

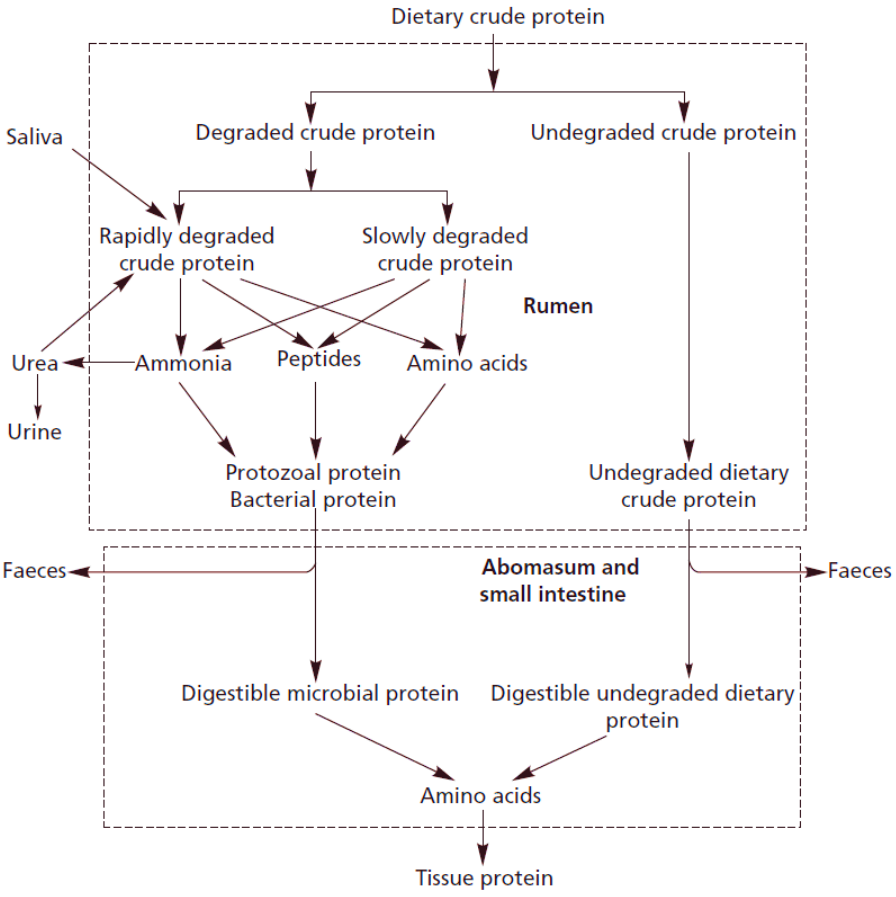


Figure 2.1 Fate of dietary crude protein in the ruminant animal (adapted from McDonald *et al*. ,2011)

Concentrate-rich diets containing sufficient levels of CP have the potential to produce the leanest carcasses (Beauchemin *et al.*, 1995). This owes to the sufficient supply of fermentable metabolisable energy from carbohydrates as well as the nitrogen from CP or NPN sources, made available to microorganisms in order to sustain microbial protein synthesis (McDonald *et al.*, 2011). It should be noted that optimal levels of growth in high producing ruminants may not be attained if the sole source of protein is from microbial origin (Stern *et al.*, 2006). Hence, in order to obtain the correct levels of amino acids required for production, the microbial protein produced in the rumen must be balanced with protein originating from the feed, which has escaped ruminal degradation (Milis *et al.*, 2007; NRC, 2007).

Beauchemin *et al.* (1995) refers to the recommendations of the National Research Council (1985a) (NRC) for early-weaned lambs, where a CP content of 14.5% in the diet is recommended for maximal growth. The amino acid requirement post-ruminally in these fast growing animals is high; however, Beauchemin *et al*. (1995) stated that the NRC (1985a) did not consider this information when it compiled the recommendations for the RUP intake requirement of lambs. The CP concentration required in the diet could be reduced by increasing the RUP supplementation, which ultimately lowers feed costs. Lambs which received supplemental RUP in their diets exhibited increased rates of weight gain and feed intake, compared to those receiving unsupplemented diets (Beauchemin *et al.*, 1995). Protein quality has been regarded as important through the different phases of growth of feedlot cattle. During the early growing period of feedlot calves, the low feed intake restricts the maximal genetic growth potential of the calf, therefore there is a greater requirement for amino acids from RUP origin (Zinn, 2014). This was demonstrated by Erickson *et al.* (2016); steer calves receiving RUP supplementation had higher ADG (0.658kg/day vs 0.576kg/day) coupled with lower FI, resulting in improved feed efficiencies when compared to those which did not receive RUP supplementation. However, during the final growing and finishing periods feed intakes are higher, thus amino acids from RDP origin are necessary in order to meet requirements (Zinn, 2014).

2.6.1 Protein quality of oilseed meals

Protein quality is a very important characteristic, which is often disregarded when various protein concentrates are considered (Dale, 1996). Erasmus *et al*. (1988) suggested the evaluation of the RUP content of a protein source upon purchase. The authors claimed that a more accurate reflection of the nutritional value of a protein source occurs because of its RUP, as opposed to its CP content.

The production of oilcake meals takes place by firstly extracting the oil from the oilseed. Two processes are commonly used in order to extricate the oil (McDonald *et al.*, 2011). These processes, however, alter the structures of the original proteins and consequently influence their nutritional value by changing their degradability properties in the rumen (Freer &Dove, 2002). The first process, the expeller process (i.e. mechanical extraction), employs high pressures and temperatures in order to extract the oil. These conditions may potentially result in protein denaturation and a reduced digestibility of the protein in the ruminant, thereby lowering its nutritive value to the animal (McDonald *et al.*, 2011). This protein denaturation can be beneficial, especially in the case of ruminant animals as a reduction in the degradability of the protein occurs. The amino acids are therefore more effectively absorbed and utilized within the small intestine. The second process is solvent extraction. This process does not use pressure in the oil extraction process, and the operation temperatures during the process are normally low, therefore the protein value of the meal produced from this process is similar to that of the original seed (McDonald *et al.*, 2011). Processing must, however, be managed and controlled properly as lysine could potentially be lost at high temperatures (McDonald *et al.*, 2011). It is thus important to consider the processing methods employed when analysing the nutritive value of various oilcake meals.

Oilseed by-products, such as cottonseed meal and soybean meal, are natural sources of protein. Stanton *et al.* (2006) stated that these natural protein sources should contribute to better performance in feedlot lambs than protein sources such as urea, blood meal or feather meal. This was confirmed in the study by Wiese *et al*. (2003); lambs receiving canola meal based diets had higher ADG values (0.272kg/lamb/day vs. 0.180kg/lamb/day) and better feed efficiency values (6.2 vs. 11.8) than those fed urea based diets. Conversely, Khan *et al*. (1997) suggested that canola meal and soybean meal were superior protein concentrates that could support lamb growth, when compared to cottonseed meal. It is important to consider the interaction between protein source and energy level supplied by the diet; these are paramount and will subsequently influence the nutritional status, performance and feed utilization of fattening ruminants (Sami *et al.*, 2010; Khalid *et al.*, 2012). If diets fed to ruminants are deficient in protein, performance, meat quality and carcass characteristics could be adversely affected (Sami *et al.*, 2010).

Varied responses can be elicited in growing animals consuming different protein concentrates, which can in turn be related to their RUP and RDP contents as well as their amino acid profiles (Khalid *et al.*, 2012). Protein concentrates which contain a higher RUP value have been proclaimed to have profound effects on growth and muscle mass accretion than those which have a lower RUP content (Khalid *et al.*, 2012). On the other hand, Stern *et al.* (2006) stated that a lack of response, with regards to higher dietary RUP supplementation,

can be attributed to inadequate MCP production within the rumen, overprotection of the protein, insufficient quantities of protein being supplied, compromised availability of amino acids in the intestine or dietary protein which possess intrinsic AA restrictions.

2.7 Bottom line

It has become evident from the literature studied that protein quality has the potential to influence the overall performance of feedlot lambs. Specifically owing to the differences in AA composition and RUP contributions of these protein sources, the outcomes could however be variable.

Dried brewers grain is a palatable feedstuff with nutrient composition which may be favourable in ruminant production, however its use globally, is on the decline. Soybean meal should possibly yield the best results in terms of the growth performance. This protein concentrate is however expensive due to its popularity as a ration component in both monogastric and ruminant feeds, which could limit its inclusion in ruminant diets. Canola meal is another promising plant protein source in South Africa due to its rapid increase in production over the past few years. Canola meal is a protein source closely comparable to soybean meal and could conceivably be an economical ingredient in feedlot rations. When considering the cost of feed production, cottonseed meal has the ability to be the cheapest option, yet can still deliver acceptable growth performance characteristics.

As mentioned before, there is limited data on the role of protein quality on the performance of feedlot lambs. The aim of this MSc study is to evaluate plant protein sources that differ in protein quality on feedlot growth performance and rumen fermentation characteristics of sheep; which will be discussed in detail in the following chapters.

Chapter 3

Materials and Methods

3.1 Introduction

The trial was conducted as two experiments: the first experiment was a feedlot growth trial, a randomised complete block design, which was conducted with 210 Döhne-Merino lambs. The second experiment was a 4×4 Latin square design in which four cannulated Merino wethers were used.

Feedlot performance parameters were measured during the feedlot growth trial, whereas digestibility samples were collected, a rumen fermentation study and an *in sacco* study were performed during the 4×4 Latin square design.

Both of the experiments were conducted on the Hillcrest Experimental Farm of the University of Pretoria, at the Small Stock Section. Approval for both experiments was issued in May 2018 by the Animal Ethics Committee of the University of Pretoria, with project number: EC026-18.

The laboratory analyses were conducted at the department of Animal and Wildlife Sciences Nutrilab, University of Pretoria and Central Analytical Laboratories (CALLabs).

3.2 Experimental diets

Four experimental diets were used in the trial. Each of the experimental diets contained a different protein source; namely: DBG, canola meal, soybean meal and cottonseed meal as the primary plant protein source. Diets were formulated on a iso-nitrogenous (14% CP on a DM basis) and iso-energetic basis (10.2 MJ ME/kg); therefore, the energy and CP content could not influence the results. All of the oilcake meals (i.e. canola, soybean and cottonseed) used in the production of the trial feed, were produced from mechanical extraction processes. The inclusion rate (as % ingredient) of DBG was higher than that of the three oilcake meals, due to the lower CP content, characteristic of this particular feedstuff.

All diets were presented in pelleted form. Two batches of feed were used in this study; the first arrived on the 6th of July and the second on the 15th of August. Both batches of feed were formulated, mixed and transported from the Tongaat Hullett Voermol Feeds mill (Main Road, Maidstone Village, Tongaat,4380, Kwa-Zulu Natal, South Africa). Both batches of feed were stored in the feed shed on the Hillcrest Experimental Farm. The feed bags from batch 1 were marked with a cross, thus avoiding confusion between bags when feeding.

The first and second batch of feed was fed during the feedlot growth trial (Experiment 1); however, the second batch of feed was exclusively fed in the 4×4 Latin square design. Both batches had ingredient compositions as shown in Table 3.1.

Table 3.1 Ingredient composition of the four experimental diets, each diet containing a different primary protein source

	Treatment[3]			
Ingredients (%)[1]	**1**	**2**	**3**	**4**
	DBG	**Canola meal**	**Soybean meal**	**Cottonseed meal**
DBG	22.20	0.00	0.00	0.00
Canola meal	0.00	10.30	0.00	0.00
Soybean meal	0.00	0.00	8.10	0.00
Cotton meal	0.00	0.00	0.00	10.20
Urea	1.23	1.23	1.23	1.23
Sugarcane Pith	14.20	20.80	21.30	19.10
Maize meal	51.80	57.60	58.70	59.10
Molasses	6.00	6.00	6.00	6.00
Ammonium Chloride	0.50	0.50	0.50	0.50
Limestone	2.10	1.60	2.20	1.90
Premix[2]	2.00	2.00	2.00	2.00

[1]Ingredients: Ingredient composition of the four experimental diets, on an “As Is” basis
[2]Premix: Trace minerals (zinc, magnesium, iron, copper, cobalt, iodine), Vitamin A and Monensin-Na (included at 18mg/kg, “as is” basis)
[3]Treatment: Specific protein concentrate included in the experimental diet
Treatment 1= Dried brewers grain, Treatment 2= Canola meal, Treatment 3= Soybean meal, Treatment 4= Cottonseed meal

3.3. Growth (feedlot) trial- Experiment 1

3.3.1 Animals and routine procedures

Two hundred and ten Döhne-Merino lambs, with average body weights of 28.3 kg (standard deviation of 4.64kg), were transported to the Hillcrest experimental farm. All the lambs originated from the same farm in the Steynsrus district of the Free State Province. Lambs were approximately 5 months of age upon arrival and were weaned the day prior to delivery to the experimental farm. The lambs, split into two large pens upon arrival, were supplied ad lib access to *Eragrostis curvula* hay and fresh water. Lambs were allowed to rest for the remainder of the day and night. Lambs were sheared the following day by a team of Cape Mohair and Wool (CMW) shearers, using machine shearers (day 2).

On the third day, lambs were subjected to routine procedures commonly practised in commercial South African feedlots. The tagging of individual lambs with a unique five-digit GMPBasic® ear tag in their left ears, took place. Lambs weights were measured using a Tal-Tec livestock scale and the recorded weights were utilized for blocking purposes as well as the allocation of animals to experimental treatments. A description of the blocking and allocation procedures follows in the experimental design (Section 3.3.2). Lambs were vaccinated against common feedlot diseases caused by *Clostridium* and *Pasteurella* bacteria, using Multivax-P Plus (MSD Animal Health, South Africa). A 2ml subcutaneous injection was administered to each lamb. A 3ml intramuscular injection of either Hi-Tet 200 L.A. Gold (Bayer Animal Health Division, South Africa) or Maxitet-LA (Cipla Agrimed, South Africa), a long acting antibiotic, was additionally administered.

A day later (day four), lambs were assigned to their respective blocks, pens and treatments as described in the experimental design (Section 3.3.2). Each lamb was tagged in the right ear with a second coloured GMPBasic® ear tag. The coloured ear tags were numbered and corresponded to specific treatment groups. The different treatments had the following ear tag colours designated to them: treatment one - white, treatment two - pink, treatment three - red and treatment four - blue. Subsequent placement of lambs in their allotted pens followed, and marked the commencement of their adaptation period to the concentrate-rich pelleted diet. The 10 heaviest lambs of the group of 210, were removed from the experiment during blocking and were placed in the “surplus pen”. This resulted in the use of only 200 lambs for the trial.

Five days later (during the adaptation period), each lamb was administered a 1ml subcutaneous vaccine in order to prevent the incidence of Scabby Mouth (Orf) (prepared by Disease Control Africa). Vaccination of lambs against Scabby Mouth ensued as an outbreak occurred amongst lambs held in the feedlot pens of the Hillcrest experimental farm during December 2017. The virus can persist in infrastructure and soil for numerous months or years following an outbreak; vaccination being a precautionary measure (Nandi *et al.*, 2011). Lambs were also given an oral dose (6ml) of Endo+Lint (Cipla Agrimed, South Africa), using a drench gun, to aid the treatment of internal parasites. No hormonal growth implants were used in the trial.

The trial commenced at the beginning of July 2018 and concluded the end of September 2018.

The feedlot growth trial was conducted for a period over 56 days. The duration of the entire trial was 82 days: four days allocated to arrival and processing, 21 days allocated for adaptation, 56 days allocated to the feedlot growth trial and 1 day allocated for slaughter.

3.3.2 Experimental design

The experimental design, was a randomised complete block design (RCB). Pens were treated as experimental units, hence the number of experimental units (i.e. 4) were identical to the number of treatments (i.e. 4) (Kaps &Lamberson, 2009). Each block contained each treatment, therefore blocks were the same size (Gomez *et al.*, 1984). Lambs were divided into five blocks, using the individual body weights recorded during the routine procedures (Section 3.3.1). Blocking animals according to weight reduces the experimental error (Kaps &Lamberson, 2009), as the variation within each block is reduced, whilst the variation amongst blocks are maximised (Gomez *et al.*, 1984). Figure 3.1 below depicts the average pen weights within each block, which are similar, whereas the pen weights amongst blocks differ. Each block consisted of 40 lambs, which were randomly assigned to one of four pens. Thus each pen contained 10 lambs. A total of 20 pens were used in the experiment as each treatment was replicated five times. Replication is required in order to estimate experimental error (Gomez *et al.*, 1984). Pens within each block were randomly assigned to one of four treatments (Kaps &Lamberson, 2009). The random allocation of animals to pens ensured that each pen contained at least five wethers and at least four ewes. The 10^{th} animal in each pen was either a ram, ewe or wether (depending on random allocation). The surplus animals were housed in pen 21. During the course of the trial, all lambs within a block were exposed to the same conditions (Kaps &Lamberson, 2009), with the exception of the treatment diets which they were fed.

Block 1

Pen 1	30.5kg	Pen 2	30.1kg	Pen 3	30.1kg	Pen 4	30.9kg
T4		T2		T3		T1	

Block 2

Pen 5	28.6kg	Pen 6	28.9kg	Pen 7	28.8kg	Pen 8	28.8kg
T1		T3		T2		T4	

Block 3

Pen 9	27.8kg	Pen 10	27.6kg	Pen 11	27.9kg	Pen 12	27.8kg
T3		T2		T4		T1	

Block 4

Pen 13	26.8kg	Pen 14	26.9kg	Pen 15	27.9kg	Pen 16	26.6kg
T1		T4		T2		T3	

Block 5

Pen 17	25.4kg	Pen 18	25.8kg	Pen 19	25.1kg	Pen 20	24.3kg
T3		T2		T1		T4	

Figure 3.1 Random assignment of treatments to pens with accompanying average pen weights, post-blocking

Treatments were as follows:

T1: Dried brewers grain (DBG)

T2: Canola meal

T3: Soybean meal

T4: Cottonseed meal

3.3.3 Adaptation period

The adaptation period serves as a time period in which lambs are allowed time to transition from a high fibre-low concentrate to high concentrate-low fibre, pelleted experimental diet (Stanton *et al.*, 2006). This period ensures the establishment of a balanced microbial population within the rumen, as well as, the hind-gut of the lambs. In addition, it allows enzyme production to adjust to the new dietary conditions in the small intestine (Bowen *et al.*, 2006).

During the adaptation period, lambs had *ad libitum* access to milled *Eragrostis curvula* hay for the first 14 days of the adaptation period, during which the quantity of pellets offered was gradually increased by 100g per lamb per day (Savage *et al.*, 2008). Inspection of pens

occurred on a daily basis during the adaptation period in order to identify lambs showing any symptoms of acute or subacute acidosis. If acute or subacute acidosis did occur, records were made of these animal numbers. The observation of acidosis under commercial feedlot conditions occurs frequently, thus, inspection is paramount as acidosis could lead to a reduction in the performance of animals (Nagaraja &Lechtenberg, 2007; Almeida *et al.*, 2018). Consequently, the adaptation period was extended due to scouring and/ or signs of sub-acute acidosis observed amongst lambs in some of the pens. Supplementation of additional hay to these pens occurred in order to assist lambs with the transition to the pelleted diets. Pens with no signs of sub-acute or acute acidosis received no supplemental hay for days 15-21; therefore, the adaptation period was a total of 21 days. Fresh water was freely available at all times.

3.3.4 Feedlot growth period

During the feedlot growth period, lambs had *ad libitum* access to their allotted experimental diets and fresh water was always freely available. Additional roughage was not provided. Each pen was allocated a specific feed bucket (with known weight), in which feed was weighed out and recorded on a daily basis (Savage *et al.*, 2008). Feed was delivered manually. Troughs were filled with fresh feed frequently during the day, so as to stimulate feed intake and achieve *ad libitum* feed supply.

Once a week (Mondays), feed samples were collected from different sections of five different feed bags, sealed in an airtight plastic bag and frozen at -20 ºC for further analyses. Upon completion of the experiment, feed samples collected from the second batch of feed were pooled using the quartering method in order to yield one representative sample per treatment (Herrman, 2001).

On the same day as which feed samples were collected, feed refusals (orts) were collected from each pen and weighed back in order to calculate feed intake. Orts from each pen were mixed thoroughly and a subsample was collected from each pen and pooled per treatment, which was subsequently sealed in a zip-lock bag and frozen at -20ºC for further analyses.

All lambs were weighed once a week, on a Tuesday morning, using a Tal-Tec livestock scale. Individual weights were recorded in order to calculate the ADG of the lambs. The same scale was used each week and animals were weighed at the same time each morning. Blocks were weighed separately. All individuals (40 lambs) from a particular block were moved to the crush section upon which they were weighed and immediately returned to their respective pens, so as to reduce stress and allow for normal feeding behaviour to continue. Weekly feed

intake values and pen weights were used to determine the FCR of each pen (Brand *et al.*, 2017).

The feedlot period was partitioned into two periods: period 1 and period 2. Refer to Figure 3.2 that outlines the dates and events of the feedlot period.

Date	Description	Period	**Feed batch fed**
6 July	**Feed batch 1 Arrival**		
7 July -10 July	Arrival & Processing		
10 July - 31 July	Adaptation	Adapt.	
31 July – 7 August	Week 1	Period 1	**Feed batch 1**
7 August -14 August	Week 2		
14 August – 21 August	-Week 3 **-15 August: Feed batch 2 Arrival**		
21 August- 28 August	Week 4	Period 2	**Feed batch 2**
28 August – 4 September	Week 5		
4 September -11 September	Week 6		
11 September- 18 September	Week 7		
18 September – 25 September	Week 8		
26 September	Slaughter		

Figure 3.2 Timeline of the feedlot experiment with respective events being outlined

3.3.5 Facility design and management

Each individual pen was equipped with an automatic continuous flow water trough; thus fresh water was freely available. Pens, separated by fences, allowed social interaction between pens. The design of the pens ensured that injuries to individual lambs were prevented. Feedlot pens had dimensions of approximately 4.5m × 13.5m, therefore ensuring a bunk space of 45cm per lamb. The provision of space at the feed trough, as well as overall pen space, was greater than that recommended for commercial feedlots. Overhead shelter extended above the feed troughs in each pen; this ensured that shade was available for lambs and in addition, protected feed from spoilage by rain. Feed and water troughs were cleaned as deemed necessary (at least once a week). Pens were inspected twice daily prior to each feeding, in order to identify metabolic disorders (such as bloat or scours) or injury. In the event that a lamb was sick and/or injured, the number was recorded and the animal received treatment accordingly. No mortalities occurred during the entire feedlot growth period.

3.3.6 Slaughtering and carcass parameters

Lambs completed 56 days on feed in the feedlot, where after lambs were subsequently weighed and the final body weight of each lamb, recorded. Lambs were not sheared prior to recording their final weights. All lambs were loaded and transported to Cavalier Abattoir (Pty) Ltd (83 Performance road, Farm Tweefontein, Cullinan, 1000), where they rested overnight.

Slaughtering of all of the lambs took place early the next morning, following approved procedures for South African abattoirs.

Lambs were electrically stunned and slaughtered according to standard abattoir protocol used in South Africa. Recording of the coloured ear tag numbers took place in the slaughtering line, prior to decapitation, in order to match Cavaliers' slaughter number to the correct carcass. Individual carcasses were inspected, underwent subsequent grading (Meat Matrix Grading V-7.8.1) as shown in Figure 3.3, and had their hot mass recorded by the abattoir. The South African Red Meat Classification System grades lamb carcasses according to their physical characteristics namely: age, subcutaneous fat depth (represented by a fat code) and carcass conformation score (Webb, 2015).

Feedlot diets containing large quantities of concentrates have the potential to negatively affect animal performance due to ruminal acidosis (Almeida *et al.*, 2018). Ruminal acidosis manifests as lesions occurring in the mucosal layer of the rumen, ruminitis and/ or liver abscesses. No eminent observations pertaining to ruminal acidosis occurred upon inspection, and as a result, the data was not considered.

Carcasses were chilled for 24 hours (4ºC), thus generating the cold carcass mass (Burger *et al.*, 2013).

Figure 3.3 Photographic image of the lamb carcass grading system, using Meat Matrix Grading at the Cavalier abattoir

3.3.7 General processing of samples prior to laboratory analyses

Initial dry matter (iDM)

Initial DM analyses were completed prior to any processing or analyses of samples in the lab. The initial DM procedure was performed following procedure 934.01(AOAC, 2000). Duplicates of initial dry matters were completed.

Drying and milling of samples

Samples were dried in tinfoil containers in a 55ºC oven for 48 hours, prior to milling. Milling of samples occurred using either a Retsch® Ultra Centrifugal Mill ZM 200 fitted with a 1mm screen, or a Retsch® Cutting Mill SM 100 fitted with a 2mm screen, depending on the requirements of the specific analysis. Samples were then placed in labelled plastic bottles, sealed and stored for subsequent analyses.

Dry matter (DM)

The DM method used in the laboratory, followed procedure 934.01 as described by the AOAC (2000). Duplicates of dry matters were completed.

3.3.8 Chemical analyses

Feed and orts

The 4 representative feed samples collected during the feedlot period (Batch 2) were used for laboratory analyses. A proximate analysis was performed on the feed samples. Orts collected during the feedlot period were not analysed since selective feeding is not possible with pelleted diets.

The completion of an initial DM analysis occurred first, where after samples were dried and milled (described in Section 3.3.7). A proximate analysis was performed on the feed samples, which included: ether extract, DM (refer to Section 3.3.7), CP, NDF, Starch, gross energy (GE), Ca- and P.

The ash content of the feed was determined directly upon completion of the DM analysis, using procedure 942.05, as described by the AOAC (2000). In addition, acid detergent insoluble nitrogen (ADIN) analyses were performed by the Central Analytical Laboratories (5 Cartwright Street, Stormill, Roodepoort, South Africa) on both batches of feed.

The CP of the feeds, as well as the raw materials, were analysed using the Leco or Dumas method as described by the AOAC (2000). The NDF content of the feeds were also

determined (ANKOM Technology method 9: Filter bag technique, ANKOM^{2000} fibre analyser; Robertson & Van Soest, 1981).

Starch concentration was analysed using the Megazyme Total Starch kit and its accompanying manual, which uses a combination of the following methods, with modifications: AOAC (Method 996.11), AACC (Method 76-13.01).

The ether extract or crude fat content of the feeds were determined by using the method of analysis 920.39 of the AOAC (2000).

Acid digestion took place prior to mineral analyses, following method 935.13 of the AOAC (2000). The concentration of the two minerals, particularly calcium, using the method described by (Giron, 1973) and phosphorus, using method 965.17 of the AOAC(2000), were analysed in all four feed samples.

An *in vitro* organic matter digestibility (IVOMD) was furthermore performed on the feed samples. The IVOMD was determined following the procedure described by Tilley & Terry (1963) as modified by Engels & Van der Merwe (1967).

The Gross Energy content of the feeds were determined by using a CAL_2K oxygen bomb calorimeter (DDS Calorimeters, CAL2K Oxygen Bomb Calorimeter, 22 Arbeid Avenue, Strydom Park, Randburg, Gauteng, South Africa).

3.3.9 Calculations and parameters measured

Performance data

Average daily gain

Individual body weights, recorded on a weekly basis, were used to determine the ADG per animal (Brand *et al.*, 2017). Accumulative as well as weekly ADG values were calculated.

$$\text{ADG(kg/lamb/day)} = \frac{BW_B - BW_A}{Days\ from\ A\ to\ B}$$

Where:

- BW_B: Body weight at day “B”
- BW_A: Body weight at day “A”

Weekly feed intake

Feed intake per pen was measured on a weekly basis. Therefore, an average feed intake per sheep per pen could be calculated. Dry matter intake was derived using the feed intake and initial dry matter results that were obtained from the laboratory analyses.

DMI = (feed delivered - remaining orts) × DM % of the diet fed (Gibb *et al.*, 2008)

Feed conversion ratio

The FCR was calculated on a “as fed” basis, using the following formula (Beauchemin *et al.*, 1995):

$$FCR = \frac{FI_{As\ Fed(kg)}}{ADG\ (kg)}$$

The FCR was additionally calculated on a “DM” basis, using the following formula (Silva *et al.*, 2016):

$$FCR = \frac{DMI(kg)}{ADG\ (kg)}$$

Weekly body weights

Body weights (BW) were recorded on a weekly basis over the duration of the trial. The total weight gained during the “on feed” period, can be calculated as follows:

$Weight\ gained_{total} = BW_{final} - BW_{initial}$

Where:

- $Body\ weight_{initial}$ represents the body weight (kg) at the start of the trial
- $Body\ weight_{final}$ represents the body weight (kg) at the end of the trial (day before slaughter)

Average pen weights were used in statistical analyses as pens were treated as experimental units.

Carcass data

Hot and cold carcass mass

Hot carcasses mass (kg) were recorded by the abattoir, shortly after dressing and hide removal. Cold carcass mass were recorded by the abattoir 24 hours post-mortem, when carcasses were chilled to 4ºC (Casey &Webb, 1995).

Dressing percentage

Dressing percentages (final live weights included fleece) were calculated using the following formula (Sheridan *et al.*, 2003):

$$Dressing\ \% = \frac{Cold\ carcass\ mass\ (kg)}{Live\ BW\ final\ (kg)} \times 100$$

Carcass classification

Lamb carcasses were classified according to the South African Red Meat Classification System, which is implemented in several South African abattoirs (Webb, 2015).

3.3.10 Statistical analysis

The model representing a randomised complete block design, is illustrated below (Kaps &Lamberson, 2009):

$y_{ij} = \mu + \tau_i + \beta_j + e_{ij}$ i = 1, 2, 3, 4; j = 1, 2, 3, 4, 5

where:

- i = 1,2,3,4: indicating the four different treatments
- j= 1,2,3,4,5: indicating the five blocks
- y_{ij} = an observation of i^{th} treatment in block j
- μ = represents the grand or overall population mean
- τ_i =represents the treatment effect of i^{th} treatment
- β_j = represents the fixed effect of j^{th} block
- e_{ij} = represents the experimental error

The data was analysed statistically as a randomised complete block design using the general linear model (GLM) of the statistical analysis system (SAS) program, in order to determine the average effects of the treatments over time. Repeated Measures Analysis of Variance with the GLM model were used for repeated period measures (SAS, 2019).

Initial mass (from period 1) were included as covariates in the models, however had no significant contribution and was subsequently omitted in the final models. Pen 19 (treatment 1, Block 5), was removed during the statistical analysis as the obscure performance of these animals did not represent realistic values. One animal from pen 18 (block 5, treatment 2) was removed during the second week of trial as it refused feed.

Means and standard error (SE) values were calculated and the Fischers test (Samuels, 1989) was subsequently used to determine significant differences ($P<0.05$) between means.

A Chi-Square (χ^2) test was completed using the SAS statistical program (SAS, 2019) in order to determine whether the different treatments had an effect on carcass classification. Differences between treatments were reported to be significant ($P<0.05$) or tended to be significant ($P<0.10$).

3.4 4×4 Latin square design - Experiment 2

Four cannulated Merino wethers, sourced from the Hillcrest Experimental farm, were randomly allocated to four experimental diets in the 4 ×4 Latin square design experiment.

The sections that follow will describe the experimental design, animals, diets and collection procedures executed during the course of the trial.

3.4.1 Experimental design

The Latin square design used, can be considered a change-over design, according to Kaps & Lamberson (2009); each treatment only appears once in each column and row, respectively. Individual columns and rows in this design, represent all of the treatments in a complete block. Three sources of variability can therefore be accounted for by a Latin square; rows, columns and treatments (Kaps &Lamberson, 2009). Each sheep subsequently received each of the treatment diets in four different experimental periods, as depicted in Table 3.2 below.

Each experimental period lasted for 15 days; 10 days for adaptation to the experimental diet and 5 days for sample and data collection (Mentz *et al.*, 2015).

Table 3.2 Experimental design of the 4×4 Latin square design depicting the allocation of wethers to treatments during different experimental periods

	Animal Number			
Experimental period	**P1302**	**P1312**	**P1309**	**P1303**
1	C	D	A	B
2	B	C	D	A
3	D	A	B	C
4	A	B	C	D

A: Treatment 1: Dried Brewers Grain (DBG); B: Treatment 2: Canola meal; C: Treatment 3: Soybean meal; D: Treatment 4: Cottonseed meal

3.4.2 Animals and management

All four wethers used in the trial had well-established rumen fistulas, fitted with rubber cannulae (Bar Diamond Inc., Box 60, Parma Idaho, USA). The wethers, approximately 5 years of age, had a mean body weight of 85.23kg upon commencement of the trial. Vaccination of the wethers with Cydectin (Zoetis ZA) took place, according to manufacturer's instructions, prior to commencement of the trial in order to reduce the internal parasitic load. Each wether was additionally administered an iron-deficiency injection (Dexiron 200, Virbac South Africa),

a multivitamin injection (Multimin®+ Se + Cu Sheep and Angora Goats, Virbac South Africa) and vaccinated against *Pasteurella* (Onderstepoort Biological Products (OBT) *Pateurella*) and *Clostridium* (Coglavax®, CEVA Animal Health) infections.

During the course of the trial, cleaning and shearing of the wool around the cannulae occurred as required, as permitted by the schedule. This was done so as to minimise the incidence of infections or blow fly strikes. The application of fly spray occurred as necessary, in order to reduce the irritation to the wethers brought about by flies. Clipping of the wethers hooves ensued prior to commencement of the trial. Wethers were inspected on a daily basis for general health.

The experimental diets were fed twice daily at 08h00 and 15h00. Providing feed two times per day achieves: improvements in synchronisation between time and feed intake (the production of acid), chewing the cud (stimulation of saliva production) and removal of end-products of fermentation from the rumen (González *et al.*, 2012). Feed intake and refusals were measured on a daily basis, so as to monitor each wethers daily intake and in order to adjust feed allocation where necessary (Du Toit, 2006). Pens were cleaned each morning and water troughs were cleaned at least every second day. During both the adaptation and collection periods, sheep had *ad libitum* access to their experimental diets and fresh water was freely available.

3.4.3 Adaptation period and facility design

The housing facility consisted of individual pens, separated by fences, thus wethers were capable of socially interacting with one another. Individual pens were equipped with continuous flow water troughs and individual feeding buckets. Pens had dimensions of approximately 1.9m × 3m. The facility was equipped with a roof and one side of the house was open in order to allow wethers exposure to natural light (normal circadian rhythm), moreover providing sufficient ventilation.

During the initial adaptation period, wethers were gradually adapted, for a period of 14 days, to the pelleted total mixed ration (TMR); transitioning from an ad libitum Lucerne-based diet to an ad libitum TMR pelleted diet. Ten days were thereafter allocated for adaptation when transitioning wethers from one experimental diet to the next, during consecutive experimental periods, (Mentz *et al.*, 2015). This would ensure that rumen microbial populations could adapt and the sheep's digestive tract could be cleared of food residues from previous diets (McDonald *et al.*, 2011) and feed intake could stabilise.

On day 7 of the adaptation period, three days prior to the commencement of sampling, wethers were weighed using a Tal-Tec livestock scale and their individual body weights

recorded. In addition, wethers were fitted with faecal bags on the same day, affording them the opportunity to become accustomed to the bags (O'Reilly, 2017). Sheep were additionally weighed after the completion of each experimental period (Du Toit, 2006).

3.4.4 Sample collection period

The sample collection period started on day 11 and ended on day 15 of each experimental period. During the five-day collection period, *in Sacco* bags were incubated and feed-, orts-, faecal- and rumen fluid samples were collected. In total, four experimental periods were completed, wherein all procedures remained constant for the duration of the trial.

3.4.4.1 Digestibility sampling

Feed and orts sampling

Feed sampling took place from day 11 until day 15 of each experimental period. On each day, one feed bag was sampled from five different locations within the bag to provide a representative sample. Samples were sealed in air-tight plastic bags and stored in a freezer at -20ºC. At the end of the experimental period feed samples were pooled, consequently yielding one representative sample per treatment per period.

The orts were sampled in a similar fashion as the feed, yielding one sample per treatment per period.

Faecal collection

Total faecal excretion was monitored during each experimental period. Faecal samples were collected from day 11 to day 15 and faecal bags were emptied twice daily, prior to each feeding (i.e. at 07:30 and 14:30 respectively) (Van Niekerk &Hassen, 2009). Upon emptying individual faecal bags, weighing of the contents took place, thus allowing the computation of the total faecal production per day. Faeces collected from both collections (per day) were thoroughly mixed and a 10 % sample was composited per treatment per wether, sealed in an air-tight plastic bag and stored at -20ºC (Sheridan *et al.*, 2003; Van Niekerk &Hassen, 2009). Faecal samples collected in each experimental period were pooled per treatment. Therefore, 16 composited faecal samples were available for laboratory analysis.

3.4.4.2 Rumen fermentation parameters

Analysing rumen fluid for its ammonia nitrogen (NH_3-N) concentration is important, as it is a key indicator of nitrogen (protein) degradation by ruminal microbes and additionally displays NPN utilization by the microbes (Broderick &Kang, 1980). Rumen fluid sampling took place from day 11 until day 14 of each experimental period for measurement of rumen pH,

volatile fatty acid (VFA) and rumen ammonia nitrogen analyses. Rumen fluid samples were collected twice daily from each wether, at specific times, as presented in Table 3.3. Rumen fluid was sampled in this fashion in order to simulate the rumen environment over a 24-hour period (Van Niekerk *et al.*, 2002).

Table 3.3 Schedule of rumen fluid collections and the sampling day on which it occurred, during each experimental period

Sampling day	Collection times	
	AM	PM
1	9:00	21:00
2	12:00	24:00
3	3:00	15:00
4	6:00	18:00

Rumen fluid was sampled from the cranial-, caudal-, ventral- and dorsal areas of the rumen and filtered through four layers of cheese cloth (Nair *et al.*, 2016). Immediately after filtration, pH of the rumen fluid was measured with a hand-held pH meter (YSI EcoSense® pH100A Meter) and recorded. Rinsing of the pH meter electrode with distilled water took place after each subsequent pH measurement. A 50ml sample of the filtrated rumen liquor was then subsampled to yield: 20ml- and 30ml samples of rumen fluid. The 20ml of rumen fluid was preserved with 4ml of a 25% H_3PO_4 solution for VFA determination (Webb, 1994). The remaining 30ml of rumen fluid was preserved with 5ml of a 50% H_2SO_4 solution for ammonia nitrogen determination (Broderick &Kang, 1980). Individual samples of rumen fluid were thoroughly mixed and subsequently frozen at -20ºC. At the end of the experiment, 128 rumen fluid samples preserved for VFA determination and 128 rumen fluid samples preserved for NH_3-N concentration.

3.4.4.3 *In sacco* bag preparation and incubation

The *in sacco* procedure commenced on day 14, after the 18:00 ruminal fluid collection time, of each experimental period and ended on day 15. The *in sacco* procedure, described by Ørskov *et al.* (1980), was followed in order to compare the CP- and DM disappearance of the raw materials at a specific time interval (16 hours) in the rumen. The time interval (16 hours) was selected, as this represents the time samples are incubated in the rumen prior to intestinal protein digestibility studies (Schroeder *et al.*, 1996; Weisbjerg *et al.*, 1996). The use of this single time point, has been used in several studies to determine relative differences in N disappearance from *in sacco* bags (Erasmus *et al.*, 1994; McNiven *et al.*, 2002; Mynhardt *et al.*, 2006).

In situ bags with dimensions of 10×20cm, and 50µm ± 10µm pore size (ANKOM Technology, 2052 O'Neil Road, Macedon, New York, 14502) were used. Two bags were prepared per animal for the 16 hour incubation time (Madsen &Hvelplund, 1994; Cruywagen *et al.*, 2003). In order to reduce the differences which exist in particle size, samples were milled prior to insertion into the bags. Concentrates are generally ground through 1.5-3mm screens, therefore the samples used in this study were milled using a Retsch® Cutting Mill SM 100, fitted with a 2mm screen (Erasmus *et al.*, 1988; Cruywagen *et al.*, 2003; Nolan *et al.*, 2010). Bags were inspected prior to any processing for holes or lesions. Bags were numbered from 1-32 with a permanent marker. Afterwards, bags were washed with luke warm water and turned inside out and washed again. All bags were dried for 24 hours in a 55 ºC oven, placed in a desiccator overnight and weighed. An initial dry matter analysis was performed on all four of the raw materials in order to determine the quantity of each raw material which should be inserted into the bags; this would be equivalent to six grams (6g) on a DM basis (Table 3.4).

After the raw materials were placed in the bags, the bags were secured with a cable tie and additionally, with a draw string, to prevent the bags from opening during incubation and losses of material occurring. All bags were prepared on the same day. Bags were tied in a line, preventing them from interfering with one another in the rumen (Ørskov *et al.*, 1980). The bags were then inserted into a 44 decitex stocking, to prevent the bags from being damaged in the rumen. Insertion of a weight into the bottom of the stocking occurred, thus preventing floatation of the bags within the rumen. Subsequent securing of the weight with a cable tie took place, so as to inhibit the weight from interacting with the bags (Cruywagen, 2006). Bags were pre-soaked in water for 1 minute, aiding rumen microbial attack of the feedstuffs upon insertion into the rumen (Michalet-Doreau &Ould-Bah, 1992). The string suspending the bags was attached to a key ring, which hung freely outside the cannula. Bags were, upon insertion, placed as deeply as possible into the rumen. The length of the string from the top bag to the cannula lid, was at least 25cm, allowing for adequate movement within the rumen (Ørskov *et al.*, 1980).

Upon removal from the rumen, bags were removed from the stocking and vigorously shaken in clean water, allowing the removal of any digesta from the exterior of the bag. The cable tie and drawstring were subsequently cut, thus debris entrapped in the creases (due to folding), could be washed away. Gentle rubbing of the bags contents between the forefinger and thumb occurred, ensuring the thorough cleansing of bags from any residual rumen contents. The bags were rinsed under cool, slowly running tap water, until the water draining through the bags ran clear (Ørskov *et al.*, 1980). Bags were sealed in an air-tight plastic bags and subsequently frozen at -20 ºC for further analysis.

Table 3.4 The bag numbers and weight of sample placed in each bag, for the *in sacco* procedure

Treatment	*In sacco* bag numbers	Weight of sample placed in bag (as is), in grams(g)
A	1,2,3,4,5,6,7 and 8	6.26
B	9,10,11,12,13,14,15 and 16	6.61
C	17,18,19,20,21,22,23 and 24	6.60
D	25,26,27,28,29,30,31 and 32	6.47

A: Treatment 1: Dried Brewers Grain (DBG)
B: Treatment 2: Canola meal
C: Treatment 3: Soybean meal
D: Treatment 4: Cottonseed meal

3.4.5 Chemical analysis

Faecal samples

Sixteen faecal samples were available for laboratory analysis, as described in Section 3.4.4.1. All faecal samples had undergone the iDM, drying, milling and DM procedures, as described in section 3.3.7, prior to any other analyses.

Additionally, faecal samples were analysed for their NDF, starch and CP content, using the same procedures described in Section 3.3.8.

In sacco bags

Bags were dried at 55ºC in an oven for 48 hours, after which bags were placed in a desiccator to cool. Post complete cooling, bags containing dried residues were weighed and their weights recorded. The dried residues were then transferred to labelled plastic bottles and sealed. The CP content of the 32 bags were analysed using the same procedure described in Section 3.3.8.

Rumen fluid samples

The 128 rumen fluid samples were analysed for their ammonia nitrogen concentration, adopting the procedure described by Broderick & Kang (1980).

Rumen fluid samples preserved for VFA analyses were not analysed due to budget constraints.

3.4.6 Calculations and parameters measured

Feed intake

Using the data obtained from the laboratory, in addition to the feed intakes measured during the trial, determination of the following parameters ensued:

- Daily measurement of the feed intake of each sheep took place during the course of the experiment. The feed intake was measured in order to monitor each wethers health; a decrease in feed intake could be indicative of illness.
- The feed intake per kg metabolic body weight ($BW^{0.75}$) was also calculated, using the wethers weight and feed intake.

Weighing of wethers occurred prior to the commencement of the collection period, upon attachment of the faecal bags. Weighing of wethers additionally took place upon completion of each experimental period.

Apparent digestibility values

The data obtained from the analyses done in the laboratory, as well as data collected during the course of the experiment, were used to calculate the apparent digestibility coefficients as follows (Silva *et al.*, 2016; Souza *et al.*, 2018):

- General formula:

  digestibility coefficient = $\frac{nutrient\ consumed(g/\ kgDM) - nutrient\ in\ faeces(g/kgDM)}{nutrient\ consumed(g/kgDM)}$

Apparent total tract digestibility coefficients were calculated for starch, CP, NDF and DM.

Degradability in sacco

The CP and DM disappearance values of the raw materials were determined after 16 hours of fermentation in the rumen, using the general formula below (McDonald *et al.*, 2011):

% N disappearance = $\frac{initial\ N - N\ after\ incubation}{initial\ N}$

For example, the disappearance of DM (DM_d) can be calculated using the following formula (Osuji *et al.*, 1993):

$DM_d = \frac{(SWi - BW) \times DMi - (SWf - BW) \times DMf}{(SWi - BW) \times DMi}$

Where:

- SW_i= initial sample weight + bag
- SW_f= residue sample weight + bag (post incubation)
- BW= empty bag weight
- DM_i= dry matter of initial sample
- DM_f= dry matter of residue sample

Rumen fermentation

Using the analyses of the rumen fluid samples collected, and pH data recorded during the experiment, the derivation of the following rumen fermentation parameters took place:

- Average and daily variation in rumen ammonia nitrogen yield
- Average and daily variation in rumen pH

3.4.7 Statistical analysis

The statistical model, which represents a Latin square design used in this experiment, is as follows (Kaps &Lamberson, 2009):

$Y_{ijk} = \mu + \gamma_i + \rho_j + \tau_k + e_{ijk}$

Where:

- i = 1 ,2 ,3 ,4: represents the four experimental periods
- j= 1 ,2 ,3 ,4: represents the four animals used
- k= 1 ,2 ,3 ,4: represents the four treatments
- $Y_{ijk=}$ Observation in the i^{th} row and j^{th} column receiving treatment k
- μ= the grand or overall mean
- γ_i= the effect of the i^{th} row (experimental period effect)
- ρ_j=the effect of the j^{th} column (animal effect)
- τ_k= fixed effect of k^{th} treatment
- e_{ijk} =experimental error

The data obtained from the 4×4 Latin square design was analysed using the GLM procedure of SAS program (SAS, 2019). Data was analysed for repeated measures whereby treatment, sheep (animal) and period effects were accounted for using the GLM procedure. Least square means and standard error values were determined. The Fischers test was used in order to declare significance between means at $P<0.05$ (Samuels, 1989).

Chapter 4

Results and Discussion

Experimental Diets

4.1 Introduction

In order to verify whether the diets were mixed properly, laboratory chemical analyses were performed on feed batch 2. The results of the laboratory analysis, performed on the feed batch, are presented in the sections that follow. The nutrient composition, the ADIN content and the visual appearance of the experimental diets will be discussed. In addition, the nutrient characterisation and lab analyses performed on the raw materials will be reviewed.

Feed batch 1 (refer to Figure 3.2) was not analysed for its chemical composition as it was fed to lambs for only a short period during the feedlot growth study (hence, its effects on the performance of the feedlot lambs have not been included in the statistical analyses). This feed batch was thus not used in the 4×4 Latin square design experiment. However, the ADIN content and visual appearance of this particular feed batch has been included in the discussion to follow, in order to motivate its omission from statistical and laboratory analyses.

Feed batch 2, discussed in the sections below, is applicable to both the feedlot growth study and 4×4 Latin square design. It is furthermore discussed in Chapter 5 and 6, as the same batch of feed was used to conduct both of these experiments.

4.2.1 Nutrient composition of experimental diets

The majority of the ingredients used in the formulation of the experimental diets are commonly used in feedlot rations (Nolte &Ferreira, 2004; Brand *et al.*, 2018). Although Lucerne hay is a popular roughage source used in many studies, the roughage source in the present study was sugar cane pith. Table 4.1 below shows the chemical composition of the experimental diets, as determined by laboratory analyses.

Table 4.1 Chemical composition of the experimental diets, mixed as batch 2

	Treatment			
Nutrients[1]	**DBG**	**Canola Meal**	**Soybean Meal**	**Cottonseed Meal**
Dry matter (g/kg DM)	894.1	895.8	891.8	903.6
Organic matter (g/kg DM) [2]	917.8	930.6	928.1	920.1
Crude Protein (g/kg DM)	147.8	140.5	146.6	148.1
Neutral Detergent Fibre (g/kg DM)	360.2	318.9	319.0	328.1
Ether extract (g/kg DM)	31.32	17.99	16.74	17.17
Starch (g/kg DM)	339.5	399.9	313.7	370.1
Ash (g/kg DM)	82.21	69.45	71.93	79.94
Calcium (g/kg DM)	14.15	11.09	10.70	12.00
Phosphorus (g/kg DM)	3.99	2.53	2.55	3.15
Ca:P ratio	3.55:1	4.38:1	4.20:1	3.81:1
Gross Energy (MJ/kg DM)	16.77	16.50	16.99	16.78
Metabolisable Energy (MJ/kg DM) [3]	10.85	11.22	11.55	11.18
In Vitro Organic Matter Digestibility (% DM)	78.87	82.95	82.89	81.28

[1]Nutrients in the experimental diets, analysed in the laboratory
[2]OM calculated using: total DM - Ash content (Faccenda *et al.*, 2018)
[3]ME values determined using the equation (Robinson *et al.*, 2004):ME (MJ/kg DM) = 0.82× (GE × IVOMD%)

The nutrient analyses (Table 4.1) confirms that the diets were formulated on an iso-nitrogenous and iso-energetic basis. Therefore, the effects of the experimental diets on animal parameters would not result due to differences in energy or protein concentration. Typical feedlot diets must contain a Calcium: Phosphorus (Ca:P) ratio of at least 1.5:1 (Freer *et al.*, 2007), which contributes to preventing the incidence of urinary calculi (Stanton *et al.*, 2006; NRC, 2007). All four experimental diets had Ca: P ratios that exceeded minimum requirements. The highest Ca: P ratios were observed in the canola meal and soybean meal diets respectively. A higher Ca value in the soybean meal diet could be attributed to the high inclusion levels of limestone in the formulation (Gibb *et al.*, 2008), as it is a feed ingredient rich in the aforementioned mineral (NRC, 2007).

The IVOMD values are in agreement with those of other concentrate feeds, as reported by Aufrere & Michalet-Doreau (1988). The slightly lower IVOMD value for the DBG treatment can be explained by its slightly higher NDF content. The starch contents of all the experimental diets can be attributed to the quantity of maize meal included in the formulation; maize meal

contains considerable quantities of easily digestible carbohydrate in the form of starch (Freer &Dove, 2002).

The NDF and EE content of the DBG treatment was higher than that of the other three treatments. This was to be expected as it has been described as a protein concentrate which naturally contains a higher NDF content (Westendorf &Wohlt, 2002) and is rich in fat (Bovolenta *et al.*, 1998). This is supported by Faccenda *et al.* (2018), who concluded that both the NDF and EE contents of experimental diets incrementally increased with higher inclusion rates of DBG.

The results in Table 4.1 thus confirm that experimental diets were properly mixed and reflects the formulation of the experimental diets as shown in Section 3.2.

4.2.2 Acid detergent insoluble nitrogen content and visual appraisal of experimental diets

Acid detergent insoluble nitrogen content

Heat damage to proteins have received considerable interest, therefore the value of a protein source can be evaluated by conducting laboratory analyses or by visually appraising the feedstuff (Schroeder *et al.*, 1996). Acid detergent insoluble nitrogen is widely used to characterise the protein in a feedstuff, which is completely unavailable for digestion and absorption in the ruminant, as it is bound to the insoluble fibre fraction of the feedstuff. The ADIN content of a feedstuff has been inversely correlated with CP digestibility (McDonald *et al.*, 2011).

All treatments from both batches of feed were subsequently analysed for ADIN contents; the results are shown in Table 4.2 and expressed as a percentage of CP.

Table 4.2 Acid detergent insoluble nitrogen content of the different protein sources for both batches 1 and 2

	Treatment			
ADIN (% CP)	**DBG**	**Canola meal**	**Soybean meal**	**Cottonseed meal**
Feed Batch 1[1]	7.57	9.44	7.65	7.47
Feed Batch 2[2]	4.73	7.95	7.17	5.14

[1]Batch 1: Feed delivered on the 6th of July, used in feedlot growth study (for the first two weeks)
[2]Batch 2: Feed delivered on the 15th of August, used in both feedlot growth and Latin square studies (refer to Figure 3.2)

The ADIN contents of the first batch of feed, for all treatments, were higher than that of batch 2. None of the ADIN values (as a % of CP) exceeded that of the maximum acceptable levels of 10-12% of CP (Van Saun, 2006).

A decline in the initial body weights of lambs consuming treatment 1 (DBG) was observed during the first few weeks (period 1) of the feedlot growth study, and subsequently, a new batch of feed (batch 2) was ordered. The visual appearance of the pellets from all of the treatments from batch 1 (Figures 4.1 to 4.4), were dark and some of pellets were extremely hard, thus the over- heating of pellets was speculated. It has been recommended by Van Saun (2006) that feeds with a dark brown colour should be analysed for ADIN content.

By incorporating a sugar-rich product, such as molasses into a feedstuff, the likelihood of the development of undesired Maillard reactions increase (Thomas *et al.*, 1998). End-products of Maillard reactions may influence the nutritional properties of carbohydrates, fibres, proteins and lipids. In the event that moisture is present during pelleting, melanoides can form due to the combination of free amino and aldehyde groups, resulting in a darker product (Thomas *et al.*, 1998). The amino acids which are usually affected by Maillard reactions include: lysine, tyrosine or methionine (NRC, 2007).

Heat treatment of brewery by-products under moist conditions, could lead to higher ADIN levels, due to the manifestation of Maillard-type reactions (Van Saun, 2006; McDonald *et al*., 2011). Dried brewer's grains are brewery by-products, therefore, the weight loss observed in animals receiving treatment 1 were assumed to be due to the excessive heat treatment of the pellets, resulting in higher ADIN fractions. From Table 4.2, it can be appreciated that the ADIN as a % of CP was not excessive. It should, however, be noted that ADIN may possibly be a poor indicator of protein damage in non-forage sources (Schroeder *et al.*, 1996). This has been illustrated in numerous studies using high-protein supplements such as soybean meal, where the ADIN was highly digestible in the lower digestive tract (Schroeder *et al.*, 1996). Therefore, all damaged protein could potentially not have been accounted for in the ADIN results. As previously mentioned, methionine is an amino acid which could become limiting during the synthesis of carcass protein (NRC, 2007). The possibility exists that this specific amino acid could, as a result, have been rendered unavailable in the first batch of feed of the DBG treatment. This could have caused the lack of growth observed in the lambs, however the reason for the weight loss still remains unclear.

Visual appraisal of feedstuffs

Pellets from both batches of feed were visually appraised and are represented in the photographic images (Figures 4.1 - 4.9) that follow.

Some pellets from treatment 1, from both batches of feed, had the tendency to crumble and become powder. The structural integrity of the pellets from treatment 1 may well have been challenged due to the high fibre content of DBG, as long fibre particles could potentially cause weak spots in the pellet, causing it to break up (Thomas *et al.*, 1998). Challenges during the pelleting of DBG mixtures were reported by Öster *et al.* (1977); disintegration of pellets occurred during mixing, consequently resulting in a fine pellet structure. Powdery bags of feed were not fed and discarded.

There were countless pellets in the bags of the three oilseed treatments which were hard and difficult to break. Pellet hardness may fluctuate, depending on the molasses content of the diet (Thomas *et al.*, 1998). Pellets which are excessively hard and durable may result in reduced feed intakes and may subsequently result in decreased nutrient utilization due to the occurrence of undesirable chemical reactions (Thomas *et al.*, 1998).

Figure 4.1 Treatment 1 (DBG) from batch 1, in the feed bag prior to feeding

Figure 4.2 Treatment 2 (canola meal) from batch 1, in the feed bag prior to feeding

The pellets from treatment 3, batch 1 (Figure 4.3) and treatment 2, batch 2 (Figure 4.7) clumped together, with that of treatment 3 being more severe and frequent in bags, when compared to treatment 2. This was of great concern as lambs risked choking if these pellets were to be consumed. Clumped pellets were removed prior to feeding, and the weight thereof accounted for in the feed intake calculations.

Figure 4.3 Treatment 3 (soybean meal) from batch 1, clumped pellets removed prior to feeding

When referring to Figures 4.4, 4.6, 4.7, 4.8 and 4.9; note the dark colour of the pellets, some of which had a glossy appearance.

Figure 4.4 Treatment 4 (cottonseed meal) from batch 1, in the feed bag prior to feeding

Figure 4.5 Treatment 1 (DBG), batch 2 in the feed bag prior to feeding

Figure 4.6 (Left) Treatment 2 (canola meal), batch 2 prior to feeding
Figure 4.7 (Right) Treatment 2, batch 2 undesirable pellets removed prior to feeding

Figure 4.8 Treatment 3 (soybean meal), from batch 2 prior to feeding

Although durability was not tested, the pellets from treatment 4, batch 2 exhibited the best structural integrity; very few pellets disintegrated in the bags, when contrasted with other treatments. These findings were corroborated by Thomas *et al.* (1998), who reported that the inclusion of cottonseed meal into feedstuffs, produced more durable pellets when compared to canola meal and soybean meal. However, some pellets with a shiny, dark appearance were also noted for this treatment (see Figures 4.4 and 4.9).

Figure 4.9 Treatment 4 (cottonseed meal), from batch 2 prior to feeding

4.2.3 Nutrient characterisation of raw materials

Table 4.3. displays the nutrient composition of the raw materials used in the formulation of the experimental diets. The RUP value of the cottonseed meal was the greatest, followed by DBG and soybean meal. Lastly, the canola meal demonstrated the lowest RUP value. This discovery, however, proves to be contradictory to the values reported in literature: the AFRC (1993) reported a greater DUP value for DBG than for cottonseed meal, which is supported by the RUP percentages supplied by Stern *et al.* (2006). As previously mentioned, due to the alternative processing methods employed by the factories producing the four by-products used, variability in the physio-chemical (including degradability) nature could be anticipated.

Table 4.3 Nutrient composition of raw materials on a dry matter basis (2019, U. Müller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380, South Africa)

	CP	RUP	NDF	ME	Ca	P
Treatment[1]		**(%CP)**		**(MJ/kgDM)**		
DBG (Barley)	27.00	51.85	46.67	12.33	0.26	0.39
Canola meal[2]	41.76	21.05	31.87	10.55	0.73	1.15
Soybean meal[2]	51.14	35.12	13.64	13.30	0.40	0.80
Cottonseed meal[2]	38.89	65.13	28.89	11.89	0.33	1.24

[1]Treatment: the primary protein concentrates included in the different experimental diets
[2]All oilseed meals were processed by means of mechanical extraction

The laboratory results of the CP and DM contents of the raw materials placed in the *in sacco* bags and incubated in the rumen, are represented in Table 4.4 below. These values were subsequently used in the computation of degradability estimates, which will be discussed in Chapter 5. The NRC (2007) classifies a feedstuff as a high protein concentrate provided that it contains more than 20% CP on a DM basis. From Table 4.4, it is evident that all four raw materials met this requirement in order to be classified as high protein feedstuffs.

Table 4.4 Chemical composition of the protein concentrates used in the experimental diets, determined by laboratory analyses

	Treatment[1]			
Nutrient[2]	**DBG**	**Canola meal**	**Soybean meal**	**Cottonseed meal**
Dry matter content (g/kg DM)	958.7	908.4	908.6	927.6
CP Content (g/kg DM)	214.2	426.8	516.6	464.2

[1]Treatment: the primary protein concentrate included in the different experimental diets
[2]Laboratory analyses of the four raw materials

The CP contents of each of the raw materials analysed, are comparable with and fall within the ranges reported in literature (Beauchemin *et al.*, 1995; NRC, 2007; McDonald *et al.*, 2011; Faccenda *et al.*, 2018). The substantial variation in the CP content of the DBG can be attributed to the alternative processing methods employed by breweries world-wide; these processing methods elicit variation in the nutrient compositions of the end-product (Westendorf &Wohlt, 2002).

Chapter 5

Results and Discussion

Feedlot Growth Trial

5.1 Introduction

In order to evaluate the effect of different protein concentrates on the growth and performance of lambs, a feedlot growth trial was conducted. The results obtained concerning growth parameters, feed intake and carcass data will be presented and discussed.

Data obtained from period one using feed from batch 1 (Figure 3.2), was excluded as the data obtained during this period was not a true reflection of the potential of the experimental diets due to mixing and pelleting issues. Data obtained from period 2 only (where feed batch 2 was fed), was analysed since batch 2 was properly mixed and pelleted.

In the literature studied, no or little information was available pertaining to the testing of all four respective protein concentrates in one single study, under South African feedlot conditions. Most of the literature referred to, therefore contained different combinations of two or three of the concentrates evaluated in the present study. It is important to take into account that the experimental diets of the studies referred to, were either formulated on a iso-energetic or iso-nitrogenous basis, but less frequently on both energy and protein.

5.2.1 The effect of different protein concentrates on the mean growth performance and feed intake of feedlot lambs

Starting live weight

The starting live weights at week 3 when period 2 commenced, differed between treatments ($P<0.05$). The lowest starting live weights (31.2kg) were recorded for animals consuming the DBG treatment, where lambs consuming the canola meal treatment, presented the highest mean starting live weight values (35.2kg). During period 1 (see Figure 3.2 and discussed in Chapter 4), numerous lambs fed the DBG treatment lost weight and additionally had low feed intakes. Blocking was however done accurately (as described in Section 3.3.2, see Figure 3.1), thus could not have contributed to the significant differences observed in the starting live weights. Hence, the differences observed could potentially be attributed to the dissimilarities of the quality of treatments from feed batch 1 and their respective effects on animal performance.

Average daily gain

The minimum mean ADG was 0.241kg/lamb/day for the canola meal treatment, and the maximum mean ADG was 0.287kg/lamb/day for the DBG treatment (Table 5.1). It is evident from Table 5.1 that the ADG of the canola meal treatment was lower ($P<0.05$) than that of the DBG, soybean meal and cottonseed meal treatments, respectively. The ADG values were lower than expected for studies under South African feedlot conditions. O'Reilly (2017) reported an ADG range, for feedlot lambs consuming alternative levels of condensed molasses solubles, of 0.271kg/lamb/day - 0.303kg/lamb/day. The prospect exists that energy supply was restricted in the present study, as Freer & Dove (2002) suggested that over a wide range of lamb weights studied, that protein deposition was to a greater extent sensitive to energy supply as opposed to amino acid supply in lambs. This could furthermore be supported by the elevated ruminal NH_3-N levels, as will be discussed in Chapter 6. Nonetheless, it should be noted that the calculated ME and CP content of the experimental diets reported by O'Reilly (2017), were higher than that of the present study, which could have contributed to the higher ADG values. This, since the ME content of the diets ranged from 11.70MJ/kg DM - 12.18MJ/kg DM, and the dietary CP content ranged from 157.0kg CP/kg DM - 163.1kg CP/kg DM (O'Reilly, 2017); evidently higher than those of this study, as shown in Table 4.1. Nevertheless, breed differences should also be considered, as the research conducted by O'Reilly (2017) used South African Mutton Merinos, which are known for their rapid growth rates (Cloete *et al.*, 2012).

Table 5.1 The effect of different protein concentrates on mean (±SE) feed intake and growth performance of feedlot lambs

	Treatment[1]				
Parameters	**DBG**	**Canola meal**	**Soybean meal**	**Cottonseed meal**	**±SE[2]**
Mean Growth Performance					
Starting live body weight (kg)	31.2[a]	35.2[b]	33.4[c]	33.6[c]	0.371
Final live body weight (kg)	43.3[a]	45.3[b]	44.7[ab]	45.5[b]	0.530
Total weight gained (kg)	12.7[a]	10.1[b]	11.2[a]	11.9[a]	0.278
ADG (kg/day)	0.287[a]	0.241[b]	0.268[a]	0.283[a]	0.007
Mean Feed Intake/Lamb/Day					
Feed intake, as is basis (kg)	1.47[a]	1.56[b]	1.55[b]	1.62[c]	0.014
Dry matter intake (kg)	1.34[a]	1.39[b]	1.38[b]	1.46[c]	0.012
Mean Feed Conversion Ratio					
FCR (as is basis)	5.20[a]	6.49[b]	5.80[c]	5.72[c]	0.147
FCR (dry matter basis)	4.65[a]	5.81[b]	5.17[c]	5.17[c]	0.131

[a,b,c] Means within rows with different superscripts differ (P<0.05)
[1]Treatment: the protein concentrate included in the different experimental diets
Treatment 1= Dried brewers grain; Treatment 2= Canola meal; Treatment 3= Soybean meal; Treatment 4= Cottonseed meal
[2]±SE: Standard error

Khan *et al*. (1997) reported ADG for Awassi lambs fed cottonseed meal, soybean meal and canola meal diets of 0.213kg/lamb/day, 0.244kg/lamb/day and 0.233kg/lamb/day, respectively. The ADG of sheep fed the cottonseed meal diets in the study by Khan *et al*. (1997) was lower (P<0.05) than that of the soybean and canola meal diets. These findings contradict the results of the present study; with a higher (P<0.05) ADG recorded for the cottonseed meal treatment when compared to the canola meal treatment (Table 5.1). Lower ADG values were also realised in the study by Khan *et al.* (1997), when compared to the present study. The slightly lower ADG values reported by the study of Khan *et al.* (1997) can be attributed to the lower CP content of the experimental diets (ca 13.6%), breed differences and a longer feeding period (90 days) to which the lambs were subjected to.

As ruminants age, protein accretion gradually decreases and reaches zero as the animal reaches mature size (Owens *et al.*, 1995). Younger lambs generally have higher levels of protein deposition, therefore greater quantities of energy is retained in the form of protein (Freer &Dove, 2002). This is due to greater water retention associated with protein deposition

(Owens *et al.*, 1995), than that of fat accretion. Fat accretion, however, increases as an animal ages and is less efficient than protein gain. Therefore, younger animals exhibit more efficient (higher) growth rates than older animals, characterised by their ADG and weight gain values.

The mean ADG values of lambs fed the DBG treatment from the current study contradict those reported by Bovolenta *et al.* (1998). For the intermediate inclusion level of DBG (20% inclusion rate of DBG: 80% Lucerne), which closely represents the inclusion level of the present study, a higher ADG of 0.366kg/lamb/day for Bergamasca lambs was reported. The higher ADG value in the study conducted by Bovolenta *et al.* (1998) can be attributed to the higher CP (182g/kg) content of the diet due to the inclusion level of Lucerne, supplementation of a protein source high in NPN and soybean-meal 200 (present in the concentrate). A combination of the aforementioned protein sources could have additionally improved the amino-acid profile supplied to the small intestine, leading to the higher ADG values as the authors stated that a positive interaction between DBG and Lucerne hay exists (Bovolenta *et al.*, 1998). Additionally, breed differences and the higher feed intake achieved by this specific breed as mentioned in Chapter 6, could have also contributed to the higher ADG values.

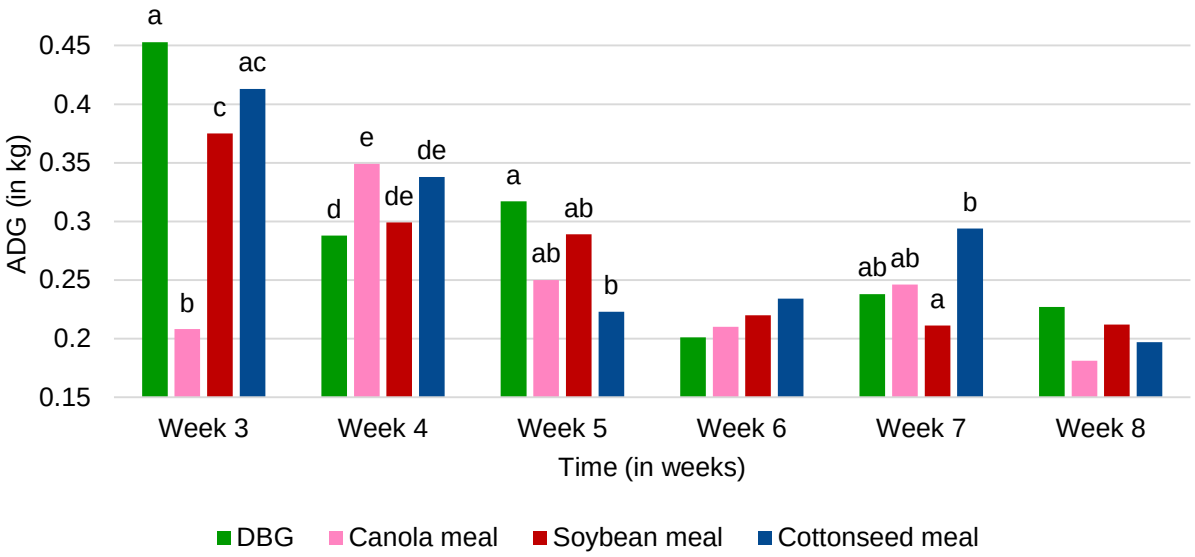
The mean ADG values reported for the canola meal treatment were slightly lower, however, still comparable to those reported by Wiese *et al.* (2003). Lambs receiving a canola-based diet had a ADG value of 0.272kg/lamb/day over a 5 week feeding period. The energy (10.5 MJ/kg) and protein content (14.4%) of the diets were virtually identical to those from the present study. The lower ADG value reported for this present study, can be attributed to the higher initial body weights of these animals, therefore less efficient growth occurring during the second period of the feedlot phase. In addition, lower feed intakes were realised in the current study, when compared to the 1.66kg/lamb/day (as is) as reported by Wiese *et al*. (2003), which could have also attributed to the lower ADG values of the present study. Additionally, a shorter feeding period of 5 weeks was used in the study by Wiese *et al.* (2003), which would inevitably impact the ADG values observed in that study.

The mean ADG values reported for the soybean meal and cottonseed meal treatments for the current trial are in contrast to the findings reported by Silva *et al.* (2016). For lambs consuming an iso-nitrogenous diet (CP content of 14%, identical to the current study), ADG values of 190g/day/lamb were reported for both the cottonseed meal and soybean meal treatments. The energy content of the experimental diets from Silva *et al*. (2016) were unfortunately not reported, and could have potentially influenced the ADG values. The animals were additionally also fed for a period of 99 days, which is longer than that of the present study. As mentioned above, older animals exhibited less efficient growth, therefore could have also influenced their respective ADG values. The NDF values of the diets fed by Silva *et al.*

(2016), were subsequently higher than that of the diets of the current study, which could have led to greater rumen fill and reduced levels of growth. It is therefore important to take various factors such as breed, diet composition, days on feed, age and environment into account when comparing results from different studies, and not to focus on ADG only.

The ADG achieved during week 3 was higher ($P<0.05$) for the DBG treatment than the canola and soybean treatments, as shown in Figure 5.1. An ADG value of 0.453kg/lamb/day was recorded for the DBG treatment during this week. Similarly, the ADG values of lambs on the cottonseed treatment (0.413kg/lamb/day), for the same week, were also higher($P<0.05$) than that of the lambs fed the canola diets (0.208kg/lamb/day). The higher ADG exhibited by the lambs consuming specifically the DBG treatment, could be attributed to compensatory growth. Refer to Chapter 4, where the negative growth performance of lambs consuming the DBG treatment was declared. This is in agreement with Brand *et al*. (2017), who reported a high initial ADG value (0.440kg/lamb/day) for South African Mutton Merinos (SAMM) fed high concentrate diets, which was credited by authors to have been due to compensatory growth.

Growth which is more rapid and efficient, is termed compensatory growth (Freer &Dove, 2002). Compensatory growth ensues when animals receive adequate quantities of good quality feed after a period of being underfed and or receiving a diet of poor quality (Freer &Dove, 2002). A higher live weight gain can be observed in these animals, when compared to those which were also adequately fed with good quality feed (i.e. with no restriction). Therefore, exceptionally high ADG for this study were not expected for the canola- soybean- and cottonseed meal treatments, respectively. It is likely that some lambs consuming the cottonseed meal treatment also experienced compensatory growth. However, no negative effects of the cottonseed meal treatment were observed in lambs fed this diet during period 1, such as formerly mentioned for the DBG treatment. The composition of compensatory growth is characterised by greater levels of protein accretion during the initial period (after receiving new or better quality feed) which is dissimilar to continuously grown animals (Freer &Dove, 2002). The rate of protein deposition in compensatory growing animals is twice, when compared to those growing normally. Proteinaceous growth contains a high water content (Freer &Dove, 2002; NRC, 2007), therefore due to the extensive protein accretion (therefore elevated water retention), live weight gain is increased in compensating individuals. It should moreover be noted that the higher ADG cannot solely be credited to higher rates of protein deposition, but can additionally be due to increased gut fill and the associated weight of the visceral organs (Owens *et al.*, 1995).



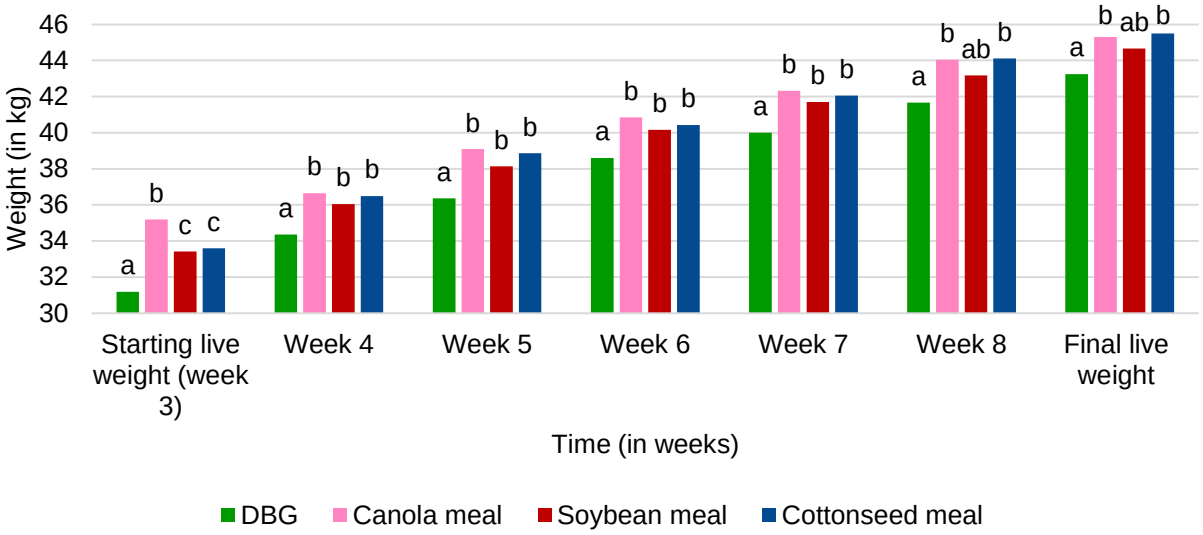
[a,b,c] Superscripts which differ above bars of individual weeks, represent differences between treatments (P<0.05)
[d,e] Superscripts which differ above bars indicate a tendency of treatments to differ (P<0.10)

Figure 5.1 Weekly mean daily gain of lambs receiving different protein concentrates in the feedlot

As days on feed in the feedlot increased, a general decrease in the ADG of each treatment were observed. This trend was also observed by Brand *et al.* (2017) and can potentially be attributed to differences in growth efficiency exhibited by animals of various ages.

Weekly weight and total weight gained

Ruminant growth is measured as the change in mass or live body weight (Owens *et al.*, 1995). Figure 5.2 illustrates that the live weight of lambs increased on a weekly basis for lambs on all treatments. It can therefore be assumed that none of the experimental diets had a negative impact on animal growth.



[a,b,c]Superscripts which differ above bars of individual weeks, represent differences between treatments (P<0.05)

Figure 5.2 Weekly mean body weight of lambs receiving different protein concentrates in the feedlot

The mean total body weight gained over the 41-day feeding period for the canola meal treatment, was lower (P<0.05) than the other three treatments. The highest total weight gained, was exhibited by lambs fed the DBG treatment, however it was not better than the weight gains exhibited by the lambs consuming the soybean meal and cottonseed meal diets, respectively (P>0.05). Results, however, should be interpreted with caution since compensatory growth, as discussed in the preceding section, could have influenced the results.

The *in sacco* study results (Chapter 6) reflected that the CP disappearance in the rumen of the canola treatment was the highest, followed by DBG, soybean meal and finally the cottonseed meal, exhibiting the lowest CP disappearance. In a review by Khalid *et al.* (2012), the authors indicated that several studies have revealed that protein sources with a higher RUP content, thus those displaying the lowest CP disappearance in the rumen, have been shown to result in greater growth rates. Lambs fed the DBG treatment exhibited the highest total weight gain, followed by the cottonseed-, soybean- and lastly canola meal treatments, respectively. From literature it is clear that level of RUP and the AA profile of RUP can affect growth performance (Erickson *et al*., 2016). The three protein concentrates with the highest protein RUP content (i.e. DBG and cottonseed meal and soybean meal), exhibited higher total weight gains, when compared to the canola meal treatment.

Final live bodyweight

The final live body weight of lambs in the DBG treatment group was lower ($P<0.05$) than that of the canola- and cottonseed meal treatments. This lower final body weight can be attributed to the growth restriction experienced by these animals during the first three weeks (i.e. period 1) in the feedlot. Freer & Dove (2002) stated that animals which have experienced growth restriction early in their lives, or for extended periods of time, may never attain the same body composition or weight of animals which did not experience restriction.

The final live body weight of animals consuming the cottonseed meal treatment was higher than that of the canola and soybean meal diets, however not significantly ($P>0.05$) so. The findings of this trial differs from that of Khan *et al.* (1997), who reported that the highest final live body weights for lambs were realised by those consuming a soybean meal treatment, followed by a canola treatment and the lowest final body weights were from lambs receiving a cottonseed meal treatment. In the present study, the higher final live body weights of the animals consuming the cottonseed meal treatment can partly be attributed to their higher DMI, which resulted in higher ADG during the feedlot period.

Feed intake

Feed intake was measured on an “as fed” basis and was subsequently converted to a DM basis, as intake on a DM basis reflects the actual nutrients consumed by an animal (McDonald *et al.*, 2011; O'Reilly, 2017). A greater prospect for elevated levels of production can be realised when animals consume more feed on a daily basis (McDonald *et al.*, 2011).

The DMI of growing lambs, as recommended by the NRC (2007), for the animals used in the present study should have ranged between 1.06kg DM/lamb/day - 1.55kg DM/lamb/day. As presented in Table 5.2, the DMI recorded over the 6 weeks for all experimental diets of the feedlot, fell well within this range.

The DMI of the DBG treatment during week 3, and subsequent weeks, was lower than expected as compensating lambs tend to consume a larger quantity of feed once the feed quality is improved. The DMI during the weeks following the provision of the new feed is in contrast with the statements of Freer & Dove (2002), who reported that the feed intake of compensating animals will be greater for a period of time, when compared to unrestricted animals.

Table 5.2 The effect of different protein concentrates on mean (±SE) weekly dry matter intake of feedlot lambs

Treatment[1]	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
DBG	1.10[a]	1.38[a]	1.33[a]	1.38[a]	1.45[d]	1.40[a]
Canola meal	1.20[b]	1.46[b]	1.37[a]	1.41[ab]	1.49[de]	1.44[a]
Soybean meal	1.18[b]	1.45[b]	1.38[ab]	1.38[a]	1.46[d]	1.44[a]
Cottonseed meal	1.24[b]	1.56[c]	1.42[b]	1.45[b]	1.56[e]	1.56[b]
±SE[2]	0.018	0.019	0.018	0.015	0.036	0.020

[a,b,c]Means within a column with different superscripts differ (*P*<0.05)
[d,e]Means within a column with different superscripts tend to differ (P<0.10)
[1]Treatment: the protein concentrate included in the different experimental diets
Treatment 1= Dried brewers grain; Treatment 2= Canola meal; Treatment 3= Soybean meal; Treatment 4= Cottonseed meal
[2]±SE: Standard Error

The mean feed intake was highest for lambs receiving the cottonseed meal diet (1.46kg/lamb/day), followed by the canola meal diet (1.39kg/lamb/day), the soybean meal diet (1.38kg/lamb/day) and finally the DBG diet, displaying the lowest intakes (1.34kg/lamb/day). The DBG and cottonseed meal diet DMI differed (P<0.05) from one another as well as from the soybean meal and canola meal diets.

The DMI of lambs consuming the DBG treatment was significantly lower than the other three treatments over feedlot period 2. Lower feed intakes of the DBG treatment can additionally be attributed to the pellet quality, as described in Chapter 4. The pellets from this particular treatment tended to disintegrate and form powder, which could have caused a reduction in feed intake. This is supported by a study conducted by Öster *et al.* (1997), who reported reduced feed intakes in young bulls fed pellets containing DBG which had disintegrated. Additionally, Bovolenta *et al.* (1998) reported lower feed intakes in lambs fed diets containing higher concentrations of DBG, and suggested that the lower intakes were due to the reduced palatability of the dried product. Similarly, in the study conducted by Öster *et al*. (1977) young bulls consumed less (0.2kg) of the DBG diet (which contained 36% DBG) during the first two months of trial. The inclusion rate of the DBG in the study by Öster *et al.* (1977) was, however, higher than that of the current trial. Nevertheless, the size of an animal, therefore its physical capacity, will influence its level of feed intake (Freer *et al.*, 2007; McDonald *et al.*, 2011). In the literature reviewed, cottonseed meal did not prove to be an unpalatable feed stuff; Kandylis *et al.* (1999) and Silva *et al*. (2016) did not detect any significant effects of increasing levels of cottonseed meal in lamb diets, correspondingly observed by Zinn *et al*. (1997) in beef steers. Thus, the higher feed intakes of this particular diet may have been credited to its palatability.

Khan *et al.* (1997) reported numerically higher DMI in Awassi lambs fed a soybean meal diet, followed by the cottonseed diet and lastly, the canola meal diet. This contrasts with the findings of the present study, where a higher (P<0.05) average DMI was observed in lambs receiving the cottonseed meal diet, followed by the canola, soybean meal and DBG diets.

Silva *et al*. (2016) reported a DMI of 1.14kg/lamb/day and 1.16kg/lamb/day for soybean meal and cottonseed meal-based diets respectively and did not differ (P>0.05) from one another (Silva *et al.*, 2016). These findings are in contrast with that of the present study; higher (P<0.05) DMI values were realised in lambs consuming the cottonseed meal treatment, when compared to those consuming the soybean meal treatment.

The lower intake of the soybean meal diet observed in the feedlot lambs, corresponds with the lower feed intakes which were additionally observed in the mature wethers, as explained in Chapter 6. It should be noted, however, that it was only 4 sheep in the 4×4 Latin square design.

The general feed intake pattern of lambs fed the four different treatments followed the same trend over time, as presented in Figure 5.3. Feed intake increased as days on feed increased after where it gradually plateaued. Brand *et al.* (2017) reported a similar trend for different sheep breeds finished off under feedlot conditions; authors anticipated that feed intake would increase with animal age and subsequently, minimal fluctuations would occur as the animal reached its mature size. This is justified by McDonald *et al*. (2011), who stated that, as animals gradually become fatter, their feed intake tends to stabilise. Thus, their intake would not continue to rise with an increase in body weight (McDonald *et al.*, 2011).

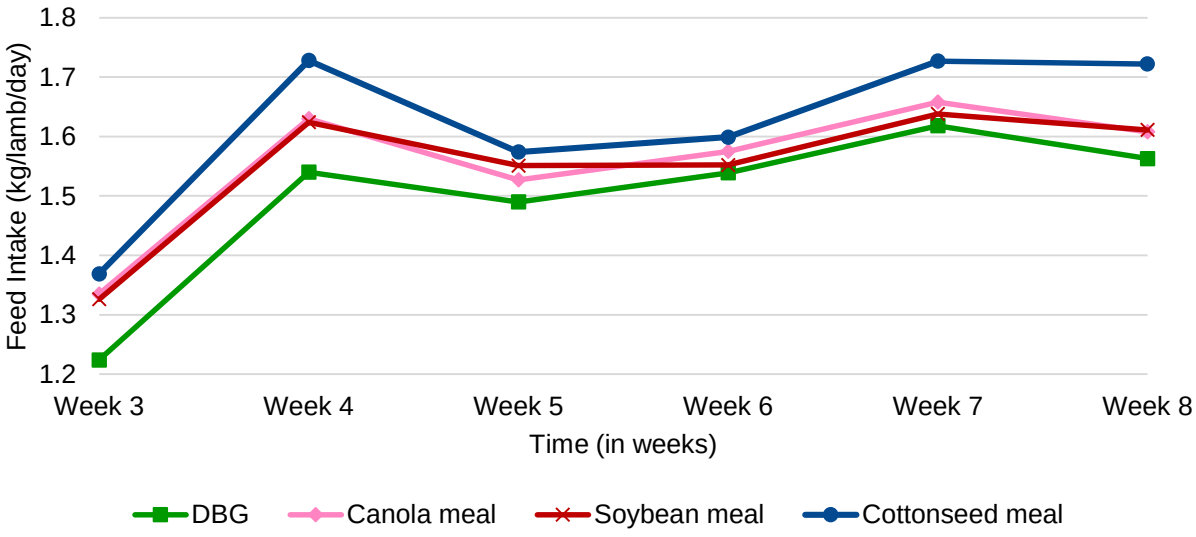


Figure 5.3 Weekly fluctuation in feed intake (as is) of feedlot lambs receiving different protein concentrates in the feedlot

No adverse weather events (rain or extreme temperatures such as a heat wave) such as declared for the 4×4 Latin square design, occurred during the feedlot growth study. Therefore, it could be speculated that the differences observed in feed intakes of different diets, between the two alternative studies, could have been influenced by weather. Additionally, animal factors such as previous experiences, can influence the intake of a specific feedstuff (NRC, 2007). The wethers used in the 4×4 Latin square design were all approximately 5 years of age, thus it can also be speculated that they have had greater exposure to different feedstuffs (in comparison to the feedlot lambs); this could have furthermore influenced their intakes of the respective experimental diets. Likewise, it is important to consider the hardness and colour of the pellets from the experimental diets, as described in Chapter 4. A great likelihood exists that Maillard reactions did take place, thus the feed intakes of all of the experimental diets could consequently have been affected. This owes to the fact that sheep are sensitive to sour, bitter, salty and sweet solutions due to their refined sense of taste (Freer &Dove, 2002).

Feed conversion ratio

One of the most important aspects in feedlot operations is feed efficiency, as it has a profound impact on the profitability of the system. Feed efficiency reflects how effectively an animal can convert the feed it consumes into product (i.e. kilograms of meat) (Khalid *et al.*, 2012). Therefore, it can be expected that animals consuming less feed, coupled with high growth rates, will resultantly have lower (more efficient) feed conversion ratios. In South Africa, feed conversion ratios serve as the measure of feed efficiency (O'Reilly, 2017). According to Bowen *et al*. (2006), several elements such as feed intake, previous nutritional background, age, live weight, genotype, sex, social interaction, disease and diet formulation will influence the feed conversion of sheep finished on high concentrate (grain) diets.

The FCR of lambs during week 3 (see Table 5.3), was significantly lower (higher numeric value) for the canola meal treatment that the other three treatments ($P<0.05$). This can be explained by the lower ADG exhibited by the lambs consuming this treatment during week three, when compared to the other three treatments ($P<0.05$).

Table 5.3 The effect of different protein concentrates on mean (±SE) weekly feed conversion ratios (DM basis) of feedlot lambs

Treatment[1]	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
DBG	2.45[a]	5.09	4.30[a]	9.87	6.17[cd]	6.60
Canola meal	5.98[b]	4.21	5.76[ab]	7.20	6.72[cd]	8.73
Soybean meal	3.24[a]	4.92	4.96[a]	7.16	7.38[c]	7.01
Cottonseed meal	3.03[a]	4.68	6.46[b]	6.53	5.36[d]	8.52
±SE[2]	0.368	0.416	0.460	1.542	0.689	1.005

[a,b]Means within a column with different superscripts differ (*P*<0.05)
[c,d]Means within a column with different superscripts tend to differ (P<0.10)
[1]Treatment: the protein concentrate included in the different experimental diets
Treatment 1= Dried brewers grain; Treatment 2= Canola meal; Treatment 3= Soybean meal; Treatment 4= Cottonseed meal
[2]±SE: Standard Error

A lower (better) mean FCR ratio (on both a DM and as-is basis) was reported for the DBG treatment than for the other three oilseed treatments (P<0.05). This was to be anticipated for these lambs, as they concomitantly had the lowest feed intakes and exhibited the highest average daily gain values over the feedlot period, when compared to the other treatments. Bovolenta *et al*. (1998) reported a feed efficiency value for a 20% DBG treatment of 197g body weight gain/kg DM consumed. This value translates to a FCR of 5.08 on a DM basis. This value is comparable, however, slightly higher (lower numeric value) than that which was reported in the current trial. The higher FCR value obtained by Bovolenta *et al.* (1998) can be explained by the higher DMI reported in the study, despite the fact that the lambs exhibited a higher ADG value.

The lambs consuming the canola meal treatment displayed a higher (P<0.05) mean FCR ratio than the other three treatments. The higher FCR of the canola meal treatment was a result of the lower ADG and higher DMI/FI reported for the lambs consuming this particular treatment, when compared to the other treatments, for the current study. Wiese *et al*. (2003) reported a feed efficiency value (kg feed as is/ kg LW gain) of 6.2 for a canola meal treatment. This value is still comparable to the slightly higher FCR reported in the current study. Although the feed intake of the animals in the study by Wiese *et al.* (2003) were slightly higher than that of the present study, the lambs exhibited a profoundly higher ADG of 0.272kg/lamb/day than the lambs from this study.

The feed conversion ratios recorded for the present study is in contrast with the findings of Khan *et al.* (1997). The authors reported feed efficiency values which were higher than those reported in the current study and were as follows; canola meal (8.58), soybean meal (8.98) and cottonseed meal (9.79), with the cottonseed meal treatment being higher (P<0.05) than those of the other two oilcake meals (Khan *et al.*, 1997). The lower (more efficient) FCR

values for the current study, for all treatments, can be justified by the higher ADG values and lower FI realised, when compared to the same parameters measured by Khan *et al*. (1997). It is also important to keep mind that Afghani lambs were used in the study by Khan *et al*. (1997) and as mentioned before, genotype or breed, can also have an impact on feed conversion values.

In the present study, no differences were detected (P>0.05) amongst the FCR values for the cottonseed meal and soybean meal treatments. These results support the findings of Silva *et al.* (2016), which reported FCR values of 6.30 and 6.46, which did not differ (P>0.05), for lambs consuming soybean meal and cottonseed meal treatments respectively. The lambs from the study by Silva *et al.* (2016) exhibited considerably lower ADG and FI values, which can explain the higher FCR values attained in the study.

5.2.2 The effect of different protein sources on carcass characteristics

The effect of treatments on hot carcass mass, cold carcass mass and dressing percentage of feedlot lambs will be discussed in the sections which follow.

Carcass characteristics measured in this trial could, however, not be compared to several of the growth or feedlot studies referred to for growth traits as characteristics such as hot carcass mass, cold carcass mass and dressing percentage were not measured (Khan *et al.*, 1997; Bovolenta *et al.*, 1998).

Hot and cold carcass mass

Hot carcass mass are recorded by abattoirs shortly after dressing and hide removal (Webb *et al.*, 2018). Carcasses are subsequently chilled for 24 hours to 4ºC, in order to yield the cold carcass mass (Burger *et al.*, 2013). Differences exist between hot and cold carcass mass as water is lost by means of evaporation from the carcasses during the first 24 hours' post-mortem, due to chilling. Greater evaporative losses occur from leaner tissue than that of fatter tissues (Savell *et al.*, 2005). Thus carcass composition could ultimately influence the price the producer receives.

The same trend in final live body weights (Table 5.1) was observed in the hot carcass mass yielded (Table 5.4). It must be noted that the highest hot carcass mass was recorded for lambs consuming the canola treatment, however they did not result in the highest final live weights. Nonetheless, the hot carcass mass of the DBG treatment was lower (P<0.05) than that of the canola and cottonseed meal treatments for the present study. This corresponds to

the lower final live weight of the lambs prior to slaughter. The hot carcass mass of the soybean meal treatment did not differ (P>0.05) from the other treatments.

Table 5.4 The effect of different protein concentrates on mean (±SE) hot carcass mass, cold carcass mass and dressing percentage of feedlot lambs

Treatment[1]	Hot mass	Cold mass	Dressing percentage
DBG	20.3[a]	19.7[a]	45.5[cd]
Canola meal	21.5[b]	20.9[b]	46.0[c]
Soybean meal	20.9[ab]	20.3[ab]	45.4[cd]
Cottonseed meal	21.2[b]	20.5[b]	45.1[d]
±SE[2]	0.191	0.195	0.320

[a,b]Means within a column with different superscripts differ (*P*<0.05)
[c,d]Means within a column with different superscripts tended to differ (*P*<0.10)
[1]Treatment: the protein concentrate included in the different experimental diets
Treatment 1= Dried brewers grain; Treatment 2= Canola meal; Treatment 3= Soybean meal; Treatment 4= Cottonseed meal
[2]±SE: Standard Error

Ponnampalam *et al.* (2005) did not detect significant differences between the hot mass of lamb carcasses produced from basal diets, supplemented with canola meal or soybean meal. Authors attributed differences recorded in carcass weights to the LW differences observed at slaughter. This is in agreement with the findings from this trial, where the hot mass of the canola and soybean meal treatments did not differ (P>0.05), however the higher hot carcass mass produced from the canola meal treatment resulted due to the higher LW at slaughter of these lambs.

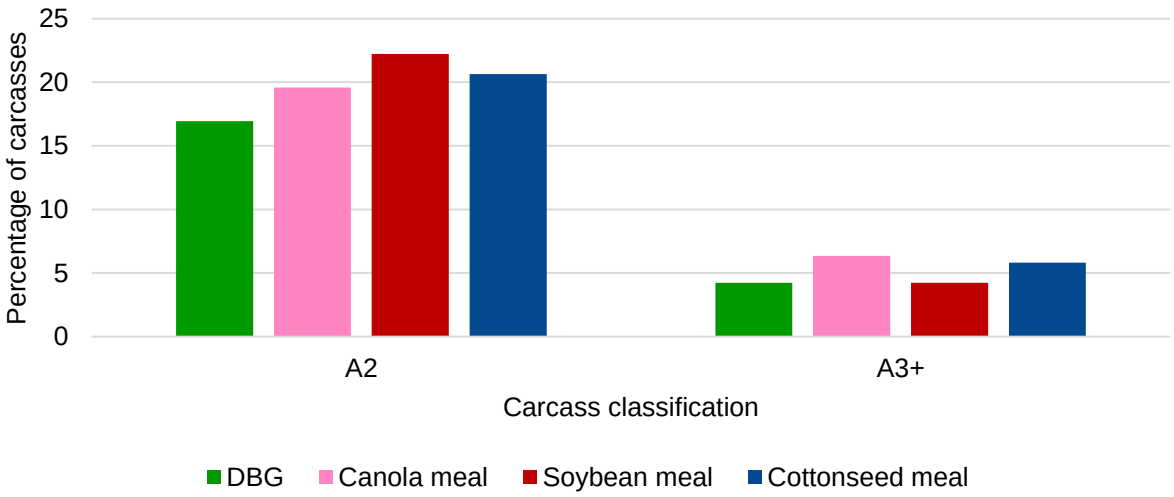
The same trend was observed when comparing the cold carcass mass between treatments, as the hot carcass mass. The cold carcass mass of the lambs receiving the canola meal and cottonseed meal treatments were higher (P<0.05) than those receiving the DBG treatment, however both did not differ (P>0.05) from the soybean meal treatment.

Silva *et al.* (2016) noticed a significant difference in the hot and cold carcass mass of lambs; lambs consuming soybean meal-based diets yielded higher hot and cold carcass mass than those consuming cottonseed meal diets, although their live weights at slaughter were similar. These findings differ from that of the present study, as no differences (P>0.05) were detected in the hot and cold carcass mass between the lambs consuming the soybean meal and cottonseed meal diets. It should be noted that the carcasses in the study by Silva *et al*. (2016), were trimmed prior to the determination of the hot carcass mass, which could have led to the differences reported.

Carcass classification

One aspect of the Agricultural Product Standards Act (Act 119 of 1990) is carcass classification; a complex system which aims to classify carcasses according to specific quality attributes which have been well defined. Unlike carcass inspection, carcass classification is not obligatory in all South African abattoirs. During carcass classification, carcasses are classified according to: age (A, AB, B or C), level of carcass fatness (fat codes which range from 0 to 6), carcass conformation (codes ranging from 1 to 5) and damage (ranging from 1 to 3) (Webb, 2015). The South African market prefers lamb as opposed to mutton; lamb being classified as animals which are slaughtered prior to tooth eruption (Cloete *et al.*, 2012), which translates to animals in age category "A" (Webb, 2015). Additionally, the South African market shows a preference for A2 and A3 carcasses (O'Reilly, 2017), which resultantly attain higher prices at slaughter (Brand *et al.*, 2018). A fat code of 4 refers to a carcass which is classified as "fat", a fat code of 3 denotes a medium fat thickness and a subsequent fat code of 2, denotes a slightly leaner carcass than the former (Webb, 2015).

The majority of carcasses produced by lambs fed the four treatment diets in this study were classified as A2 (79.37 % of all carcasses), followed by A3+ (20.63% of all carcasses), as shown in Figure 5.4. There were no (P>0.05) differences detected in the Chi-Square test between treatment and the carcass classification which they yielded. This is supported by Beauchemin *et al.* (1995), who argued that diet formulation has a reduced ability to affect carcass leanness when lambs consume concentrate-rich diets and are slaughtered at live weights which are similar.



A3+: denoting carcasses classified as A3 & A4, with the former contributing to the majority

Figure 5.4 Carcass classification of feedlot lambs fed different protein concentrates

Alternatively, Öster *et al.* (1977) found a significantly lower (P<0.01) fat deposition in the carcasses of beef bulls which were fed diets containing a high (36%) DBG content. However, the carcass classification system used in the United States of America (USA) differs from that used in South Africa, with accompanying differences in consumer preferences for carcasses (Webb, 2015). Wiese *et al.* (2003) stated that canola meal-, lupin- and urea-based diets all produced carcasses acceptable to the Australian market. Unfortunately, the authors failed to describe “acceptable carcasses”, therefore comparisons to the canola meal treatment of the present study could not be made.

Carcass classification were not reported by Silva *et al.* (2016), Khan *et al.* (1997) or Bovolenta *et al.* (1998), hence analogies could not be made.

Dressing percentage

The gut contents and skin (fleece) weight of animals influence their final live-weight prior to slaughter, therefore affecting dressing percentage (Cloete *et al.*, 2012). This is supported by Beauchemin *et al.* (1995) who stated that wool growth has the potential to influence dressing percentage.

The average dressing percentage of the current trial was 45.53%, at an average final live-weight of 44.99kg. Sheridan *et al.* (2003) reported dressing percentages of SAMM lambs, receiving either low or high energy feedlot diets, of 45.61% (at a final LW of 43.51kg) and 50.14% (at a final LW of 49.05kg), respectively. The energy content of the diets of the current study was an intermediate to the high energy diet ME content (12.11MJ /kgDM) and the low energy diet (9.89 MJ/kg DM) tested by Sheridan *et al*. (2003). Nevertheless, it is important to note that SAMM is a later-maturing breed which exhibits higher live weights at slaughter (due to faster growth rates) than the Döhne-Merino (Cloete *et al.*, 2012), which explains the slightly higher dressing percentages reported for the SAMM lambs. Correspondingly, Beauchemin *et al.* (1995) credited lower dressing percentages in lambs to lower live weights at slaughter.

The results from the present study are conflicting with results obtained by Cloete *et al*. (2012). Carcass traits of several sheep breeds, common in South African production systems, were compared to each other and it was confirmed that sheep (20 months of age) from the Döhne-Merino breed yielded dressing percentages of 40.5% with a corresponding slaughter weight of 56.0kg. The animals used in the study by Cloete *et al*. (2012) were older than the animals used in the present study (approximately 7-8 months of age at slaughter) and were kept on pasture. A higher dressing percentage would have been expected for the more mature animals of the study by Cloete *et al.* (2012), owing to the fact that, as live-weight and level of fatness increase, so does the dressing percentage (Owens *et al.*, 1995; Cloete *et al.*, 2012). However, Owens *et al.* (1995) stated that dressing percentage increases when diets rich in

concentrates are fed, as opposed to roughage diets. High roughage diets lead to increased gut fill, resulting in a reduction in dressing percentage (Sheridan *et al.*, 2003). Therefore, this explains the higher dressing percentage reported in the current study; diets containing less roughage could potentially lead to a reduced gut fill, leading to higher dressing percentages.

The dressing percentage of the canola meal treatment was the highest out of all of the four treatments. The lowest dressing percentage for this study was reported for the cottonseed meal treatment. The dressing percentages from the canola meal and cottonseed meal treatments in this study tended to differ from each other ($P<0.10$). The lower dressing percentage of the cottonseed meal treatment may perhaps be related to the marginally higher NDF content of the diet, which attributed to a greater gut fill in the lambs. This is in agreement with Silva *et al.* (2016), who reported lower dressing percentages for lambs consuming a cottonseed meal treatment when compared to a soybean meal based diet, with some researchers attributing the lower dressing percentages to the higher NDF content of the cottonseed meal-based diet. Alternatively, the differences observed in dressing percentages could have been influenced by fleece weight, as animals were not sheared prior to slaughter, thus dressing percentages reported for the current study were not fleece free.

At a final live weight of 45.2kg, Wiese *et al.* (2003) reported a dressing percentage of 45.5% for lambs consuming a canola meal-based diet. This lower dressing percentage is still comparable with the dressing percentage of the lambs from the canola meal treatment from the current study.

Öster *et al*. (1977) reported a significantly lower dressing percentage for young bulls which consumed diets containing a higher DBG (36%) content, when compared to a soybean meal and alternative DBG diet with lower DBG content (17%). The soybean meal diet yielded the highest dressing percentage out of the three treatments (Öster *et al.*, 1977). However, the authors attributed the lower dressing percentage of the high DBG diet to the leaner carcasses of animals which consumed this treatment. These results are conflicting with the findings of the current trial, as the dressing percentage of the DBG and soybean meal diets did not differ from each other ($P>0.05$).

Chapter 6

Results and Discussion

4×4 Latin Square Design

6.1. Introduction

In order to investigate the effect of the four different primary dietary protein sources on the intake, digestibility, rumen fermentation and N disappearance (at 16 hours) in sheep; a 4×4 Latin square design trial was conducted. The results obtained from the 4×4 Latin square design trial will be presented and discussed in the sections that follow.

Limited studies exist for both sheep and cattle, which compare all of the aforementioned parameters, specifically for concentrate-rich feedlot diets containing the protein concentrates of interest. This due to the fact that the majority of studies used in the discussion (for comparative purposes), provided high roughage basal diets. These basal diets included various types of silages, grasses or hays (straws), which were supplied with a protein concentrate supplement in which a specific protein source contributed profoundly, not always exclusively to the CP content of the diet. Additionally, some diets were formulated on an iso-nitrogenous or iso-energetic basis, but infrequently on both.

6.2 The effect of different protein concentrates on the dry matter intake and nutrient digestibility of mature cannulated wethers

It has been suggested that the intake of diets, such as provided in feedlots, can be limited by conditions in the rumen (acid load, osmolality, pressure and absorption) as well as nutrient absorption and usage (Owens *et al.*, 1995). Table 6.1 below illustrates the feed intakes of mature wethers fed the experimental diets. By expressing DMI as a function of metabolic body weight, the differences in body weight of wethers are taken into account.

Table 6.1 The effect of protein concentrate on mean (±SE) dry matter intake, dry matter intake per kilogram metabolic body weight and total tract apparent nutrient digestibility in sheep

	Treatment[1]				
	DBG	**Canola meal**	**Soybean meal**	**Cottonseed meal**	**± SE[2]**
Intake					
Average DMI (g/day)	1363.4[a]	1215.8[ab]	1119.9[b]	1152.0[ab]	64.821
Average DMI (g/kgBW$^{0.75}$)	47.28[c]	42.84[cd]	39.67[d]	40.75[cd]	2.629
Digestibility (%)					
DM	67.51[c]	71.45[cd]	73.57[d]	72.67[cd]	2.032
CP	77.31[a]	77.01[a]	81.18[b]	78.52[ab]	1.036
Starch	99.82	99.77	99.85	99.86	0.039
NDF	43.78	46.68	48.64	47.82	3.513

[a,b] Means within the same row with different superscripts differ (P<0.05)
[c,d] Means within the same row with different superscripts tended to differ (P<0.10)
[1]Treatment: the protein concentrate included in the different experimental diets
Treatment 1= Dried brewers grain; Treatment 2= Canola meal; Treatment 3= Soybean meal; Treatment 4= Cottonseed meal
[2]±SE: Standard Error
DMI: Dry Matter Intake
BW$^{0.75}$: Metabolic body weight
DM: Dry Matter
CP: Crude Protein
NDF: Neutral Detergent Fibre

Higher (P<0.05) daily dry matter intakes were realised on the DBG diet in mature wethers, when compared to the soybean meal treatment. This contrasts with the findings of Faccenda *et al.* (2018), which did not detect any significant differences in the DMI of cattle consuming soybean-meal only (no DBG supplementation) and DBG only diets. The CP contents of the experimental diets were slightly lower (ca. 12%) than those of the current study. Nonetheless, the failure of Faccenda *et al.* (2018) to detect a difference could be attributed to the fact that corn silage contributed to a considerable part of the ration (included at a rate of 50%); silage is a food source which characteristically has a variable composition and can contain interacting factors which may influence intake. These factors include: pH, fibre (g/kg DM) and concentrations of organic acids (g/kg DM) but to mention a few (McDonald *et al.*, 2011). Another probable reason for the differences observed, is that the soybean meal diet contained the highest inclusion rates of limestone, which can potentially justify the lower dry matter intakes of this treatment. Gibb *et al*. (2008) reported lower DMI in feedlot cattle receiving a diet supplemented with limestone, which was practised in order to improve the Ca: P ratio.

A tendency (P<0.10) of the DMI per kg of metabolic body weight ($BW^{0.75}$) to be higher on the DBG diet, when compared to the soybean meal diet, was also detected and support the DMI(kg/day) data. Bovolenta *et al.* (1998) reported the DMI of lambs as a function of metabolic weight for the DBG_{20} diet (similar to the DBG inclusion rate of the present study) of 125g/kg $BW^{0.75}$/day. This figure is much higher than realised in the present study, and can be attributed to the breed used in the Bovolenta *et al.* (1998) study, the Bergamasca. Authors reported that this breed is renowned for its ability to realise high feed intakes.

Environmental conditions, such as weather, can have a major impact on the feed intake of ruminants (McDonald *et al.*, 2011). Adverse weather was experienced during some of the collection periods of the 4×4 Latin square study. Thunderstorms transpired on some of the collection days, and although temperatures were not recorded, a heat wave occurred in Pretoria during the week of 12-17 November 2018, when the trial was still in progress. When animals are exposed to elevated environmental temperatures, DMI generally decrease and it is therefore important to note that environmental factors could have influenced the feed intakes observed in the wethers.

The true value of a specific feed source to an animal is indicated by its digestibility; McDonald *et al*. (2011) states that this is the proportion of feed which is presumably absorbed, and hence, not eliminated in the faeces. In Table 6.1 is shown the apparent total tract DM-, CP-, Starch- and NDF digestibility coefficients of the four respective experimental treatments.

The DM digestibility of the DBG treatment tended (P<0.10) to differ from the soybean meal treatment. This is in agreement with the findings of Faccenda *et al.* (2018), who detected a linear (P<0.05) decrease in the DM digestibility of soybean meal diets which contained incrementally higher levels of DBG. The reduced digestibility of DBG diets was attributed to the NFC fraction which is more resistant to ruminal degradation; owing to the extensive loss of starch from this fraction during the malting process of beer production (Faccenda *et al.*, 2018).

Both Silva *et al.* (2016) and Milis *et al.* (2007) reported higher (P<0.05) DM digestibility coefficients of soybean meal diets when compared to cottonseed meal based diets. This contradicts the findings of the current study, as no differences (P>0.05) were detected for the digestibility coefficients of these two particular diets. The lower digestibility's from the study by Silva *et al.* (2016) were accredited to the higher lignin contents of the cottonseed meal experimental diets. Additionally, the lower DM digestibilities reported by Milis *et al.* (2007), could be as a result of the higher NDF concentration of the cottonseed meal-based experimental diets.

A difference (P<0.05) was detected between the CP digestibility of the soybean meal treatment and both the DBG and canola meal treatments; the soybean meal diet resulted in a higher CP digestibility coefficient than the other two treatments. On the contrary, Faccenda *et al*. (2018) did not detect any differences in the CP digestibility of soybean-meal based diets, which had incremental higher inclusion rates of DBG. Similarly, Zagorakis *et al*. (2015) found that the CP digestibility of soybean- and canola meal supplemented diets, did not differ from one another (P>0.05). For the present study, a lower (P<0.05) DMI was reported for the soybean meal diet when compared to DBG diet. Although not different (P>0.05), a higher DMI was also observed for the canola- when compared to soybean meal diet. It has been accepted that, when the amount of food consumed by an animal increases, so does the rate of passage (AFRC, 1993; McDonald *et al.*, 2011). Consequently, faster passage rates reduce the time feed particles are subjected to the action of digestive enzymes, ultimately reducing the digestibility of the feedstuff (McDonald *et al.*, 2011). Hence, due to the lower feed intake of the soybean meal diet, feed particles could have been digested to a greater extent, which could justify the higher CP digestibility observed.

The CP digestibility coefficients of the soybean- and cottonseed meal treatments did not differ from one another (P>0.05) which is consistent with results reported by Silva *et al.* (2016).

The total tract digestibility of starch remained unaffected (P>0.05) by treatment in the current study. This is consistent with the results of Zinn *et al.* (1997) which did not identify any treatment effects of feedlot diets containing alternative levels of cottonseed meal, on the total tract starch digestion of beef steers(P>0.10). However, it is important to note that the digestible carbohydrate fraction of ruminant feeds is generally overestimated. This is because methane is an end-product of carbohydrate fermentation in the rumen, which is expelled by eructation, and is thus not absorbed (McDonald *et al.*, 2011). Nonetheless, the apparent digestibility of starch along the entire digestive tract has been suggested to be 90-100% of starch intake (Huntington *et al.*, 2006), which agrees with the results of the present study.

No differences were detected in the NDF digestibility of the four experimental diets used in the present study (P>0.05). These results conform to those reported by Faccenda *et al.* (2018); the authors detected no significant differences in the NDF digestibility of soybean-meal based diets which contained alternatively higher levels of DBG supplementation. Correspondingly, Zagorakis *et al*. (2015) discovered no difference in the NDF digestibility of experimental diets containing either canola meal or soybean meal (P>0.05).

Contrary to the outcomes of the current study, differences (P<0.05) were identified by Silva *et al.* (2016) and Milis *et al.* (2007) between the NDF digestibility coefficients of soybean and cottonseed-meal supplemented diets respectively. Soybean meal resulted in higher NDF

digestibility coefficients in both of these studies. The authors from the Silva *et al.* (2016) study hypothesised that the differences in the digestibilities which were observed, were as a result of the quality and quantity of the NDF fraction originating from the cottonseed meal. However, digestibility estimates can be influenced by the diet form. Feed with a reduced particle size such as in a meal or pelleted form, have a faster rate of passage than those with an increased particle size, such as forages (McDonald *et al.*, 2011). Opposed to the current study, the roughage sources used in the aforementioned studies were not processed. Alfalfa hay and Tifton 85 grass were incorporated in the rations reported by Milis *et al*. (2007) and Silva *et al.* (2016) respectively, whereby the physical form of these roughages and their effects on digestive processes could have potentially influenced digestibility coefficients.

Ultimately, owing to the chemical variability of oilcake meals (Silva *et al.*, 2016) - which fluctuate with the method of extraction employed (mechanical or chemical), the cultivar and processing efficiency at the extraction plant - the differences in results of digestibility coefficients between alternative studies could have been anticipated.

6.3 The effect of different protein concentrates on the 16h *in situ* ruminal crude protein and dry matter disappearance

It is important to note that a complete degradability study, such as described by Ørskov *et al.* (1980) - who made use of multiple time intervals (at which *in sacco* bags were removed), regression curves and in addition, computed the effective degradability of protein and dry matter at different outflow rates - was not applied to this specific study. The ultimate goal was to determine the relative differences between DM and CP disappearance of the four respective protein concentrates at the 16-hour time point. This specific time point has been selected as it simulates the retention time of feed in the rumen (Paz *et al.*, 2014) and has been used in numerous ruminant studies (McNiven *et al.*, 2002; Mynhardt *et al.*, 2006). Previous studies, in which DM and CP degradability estimates were provided for the 16-h incubation time or at outflow rates of $k=0.05h^{-1}$, were selected to compare with the findings of the present study. This as the AFRC (1993) recommends an outflow rate of 0.05/h for sheep consuming less than twice of their maintenance requirements, yet are at elevated planes of feeding. Additionally, this outflow rate is relevant, as all diets in the present study were in a pelleted form. The flow rate of liquids from the rumen of animals consuming concentrate diets, as opposed to high fibre diets, is slower owing to less rumination, thus reduced saliva production (McDonald *et al.*, 2011; González *et al.*, 2012). According to McDonald *et al*. (2011), the solubility of a protein source in the ruminal fluid is reflected by its nitrogen disappearance from *in sacco* bags, which is equivalent to degradability. Thus, the results presented in Table 6.2 can be assumed to be estimates of degradability of the protein sources at the 16h time point.

Table 6.2 The effect of protein concentrate on the mean (±SE) ruminal *in situ* dry matter and crude protein disappearance at 16 hours in sheep

Treatment[1]	DM disappearance	CP disappearance
	%[2]	
DBG	33.32[a]	57.71
Canola meal	60.98[b]	66.11
Soybean meal	67.34[b]	56.88
Cottonseed meal	54.89[b]	54.25
±SE[3]	4.109	5.431

[a,b] Means within the same column with different superscripts differ (P<0.05)
[1]Treatment: the protein concentrate included in the different experimental diets
Treatment 1= Dried brewers grain; Treatment 2= Canola meal; Treatment 3= Soybean meal; Treatment 4= Cottonseed meal
[2]Calculated as % (both CP and DM): [(initial - residue post 16h incubation) / initial] × 100; according to Erasmus *et al.*, 1994
[3]±SE: Standard Error

The DM disappearance of the DBG was lower (P<0.05) than that of the other three protein concentrates. Armentano *et al.* (1986) similarly reported undegraded DM values in lactating Holsteins of 0.61 and 0.18 for DBG and soybean meal respectively. Hence, the authors proposed that ruminal microorganisms were virtually unable to digest a substantial quantity of the DM of DBG (Armentano *et al.*, 1986).

Milis *et al.* (2007) reported a higher (P<0.10) effective DM degradability of soybean meal (0.56) in ewes, when compared to cottonseed meal (0.31) at an outflow rate of $k=0.05h^{-1}$. This is conflicting with the results of the present study, as no differences (P>0.05) were detected in the DM disappearance when these two protein concentrates were compared.

Paz *et al.* (2014) reported differences (P<0.01) in DM degradation of canola meal (68.6%) and soybean meal (78.9%) after 16 hours of incubation. Although differences (P>0.05) were not detected between the DM disappearance of the protein concentrates in the current study, a similar trend was observed. The values reported by Paz *et al.* (2014) were slightly higher than that reported for the present study; nevertheless, a marginally higher DM degradation for soybean meal, when compared to canola meal, was reported in the current study.

No differences (P>0.05) were detected in the CP disappearance amongst the four protein concentrates for this study. The protein concentrate which had the highest CP disappearance was the canola meal, followed by DBG, soybean meal and lastly, the cottonseed meal, which exhibited the lowest CP disappearance. Owing to the higher RUP content, as reported in several studies for DBG (Westendorf &Wohlt, 2002; Stern *et al.*, 2006), a much lower CP disappearance would have been expected for the DBG treatment than what

was found in this study. This is supported by the findings of Armentano *et al.* (1986), which reported a greater proportion of undegraded nitrogen for DBG, when compared to soybean meal. However, from the nutrient composition of the raw materials used in the formulation of the experimental diets (Table 4.3), the RUP value of the DBG was lower than that of the cottonseed meal, which clarifies these observations.

No ($P>0.05$) differences were detected in the CP disappearance estimates of the canola and soybean-meal in the present study. Paz *et al.* (2014) reported non-significant differences in the values of 75.7% and 68.8% for canola meal, and soybean meal respectively. The results from the present study are lower, yet still comparable with the findings of Paz *et al*. (2014); higher CP disappearance rates were realised for canola meal as opposed to soybean meal. The lower CP disappearance estimates reported in the present study could potentially be attributed to alternative processing methods employed by the factories manufacturing these by-products. For example, in the production of the canola- and soybean meal, higher temperatures could have been applied in the process by the factories in South Africa, as opposed to those in Canada. As the oilseed meals used in the present study were produced by means of mechanical extraction (2019, U. Müller, Pers. Comm., Voermol, P.O. Box 13, Maidstone, 4380, South Africa), the high temperatures employed during this extraction process could have denatured some of the protein, and consequently reduced the protein degradability in the rumen, thereby, reducing the CP disappearance from the *in sacco* bags in the current study (McDonald *et al.*, 2011).

Milis *et al.* (2007) detected a tendency for a higher ($P<0.10$) effective protein degradability (EPD) at outflow rates of $k=0.05h^{-1}$ for soybean meal, when compared to cottonseed meal. This is conflicting with the results from the present study, as no statistical significance was detected for the CP disappearance of these two respective raw materials. It should be noted that in the study by Milis *et al.* (2007), concentrate mixtures and not the raw materials exclusively, were incubated in the Dacron bags, which could have impacted the results.

Nevertheless, the high standard error, thus large variation in the results from the present study, could be the reason why no significant differences were detected in the CP disappearance between the four protein concentrates. Additionally, microbial contamination (MC) of the residues within incubation bags were not measured during the present study. Microbial contamination of bag residues, of which the majority is usually removed during washing (Paz *et al.*, 2014), can however lead to large variations in RUP data for identical feeds (Milis *et al.*, 2007).

6.4 The effect of different protein concentrates on rumen fermentation

Both ruminal pH and NH_3-N concentration have been described as indicators of the stability of the ruminal environment (Freer *et al.*, 2007). Table 6.3 shows the average rumen pH and average rumen NH_3-N concentrations measured over the four experimental periods.

Table 6.3 The effect of protein concentrate on mean (±SE) ruminal pH and ruminal ammonia nitrogen concentration in sheep fed four different protein concentrates

Treatment[1]	Average rumen pH	Average rumen NH_3-N concentration (mg/100ml)
DBG	5.69[a]	28.5
Canola meal	5.77[a]	27.1
Soybean meal	5.70[a]	24.4
Cotton meal	6.03[b]	22.6
±SE[2]	0.066	2.537

[a,b] Means within the same column with different superscripts differ (P<0.05)
[1]Treatment: the protein concentrate included in the different experimental diets
Treatment 1= Dried brewers grain; Treatment 2= Canola meal; Treatment 3= Soybean meal; Treatment 4= Cottonseed meal
[2]±SE: Standard Error

Rumen pH

Healthy ruminal function is subjective to pH and is vital for the maintenance of microbial populations and their associated products of fermentation, as well as ensuring the physiological functioning of the rumen (motility and absorption). Cattle consuming diets rich in concentrates, typically have a limited capability of buffering the rumen due to the decreased rates of rumination and mastication, which advertently reduces salivary production (Nagaraja &Lechtenberg, 2007).

The mean rumen pH for the current study ranged from 5.69 - 6.03. The cottonseed meal treatment exhibited a higher (P>0.05) average rumen pH than that of the DBG-, canola meal- and soybean meal treatments, respectively. Wanapat *et al.* (2012) reported lower ruminal pH values for low cottonseed meal (LCM) diets, when compared to high cottonseed meal (HCM) diets. They attributed these differences to the alternative rates of fermentation in the rumen, as the RUP characteristics of these two protein sources varied. The CP of the different protein concentrates used in the formulation of the experimental diets for the present study, as mentioned before, have dissimilar solubility characteristics in the rumen, which will consequently influence its accessibility ruminal microorganisms (Freer &Dove, 2002). It can therefore be assumed that the protein source which contributed the least to this pool, would exhibit higher rumen pH values. This would be due to lower rates of microbial growth, thus

lower rates of microbial fermentation. For the present study, this result would have been anticipated for the DBG treatment, as it has been reported in literature to have a low RDP contribution, which was not observed in the pH data (Westendorf &Wohlt, 2002; Stern *et al.*, 2006). However, from the *in sacco* study results, as presented in Section 6.3, the CP disappearance of the DBG was higher than anticipated. Despite the contrary, cottonseed meal was the oilseed meal used in the present study with the highest RUP content, according to literature (Nagalakshmi *et al.*, 2003b; Stern *et al.*, 2006; Wanapat *et al.*, 2012). Furthermore, the cottonseed meal contained a greater RUP (Table 4.3) and exhibited the lowest CP disappearance from the *in sacco* bags (numerical, not significant). It is more likely that less fermentation occurred in the rumen due to lower quantities of protein being available to microbes, thus resulting in the higher pH values observed, for the cottonseed meal treatment. Unfortunately, the VFA concentration and proportions thereof yielded in the rumen by the experimental diets, were not evaluated in the present study, thus could not be used to verify the suggested microbial fermentation rates. Nonetheless, as mentioned in Chapter 4, the pellets of the DBG diet showed a tendency to disintegrate and become powder. Feeds which have a fine structure may subsequently lead to accelerated rates of fermentation in the rumen and resultantly, cause the ruminal pH to decrease (NRC, 2007). This could as a result explain the lower ruminal pH values observed for the DBG treatment.

Figure 6.1 represents the ruminal pH values which were recorded, corresponding to the rumen fluid sampling schedule. During the course of the day, feed intake and chewing (two aspects of feeding behaviour), closely relate to the fluctuations in rumen pH (González *et al.*, 2012). Fluctuations in ruminal pH also transpired, during the course of the day, for the present study for all experimental diets. Previous studies reported that a decrease in ruminal pH occurs post-feeding, whereby it gradually recovers (Nagalakshmi *et al.*, 2003b; Faccenda *et al.*, 2018). This general pattern was also observed in the present study.

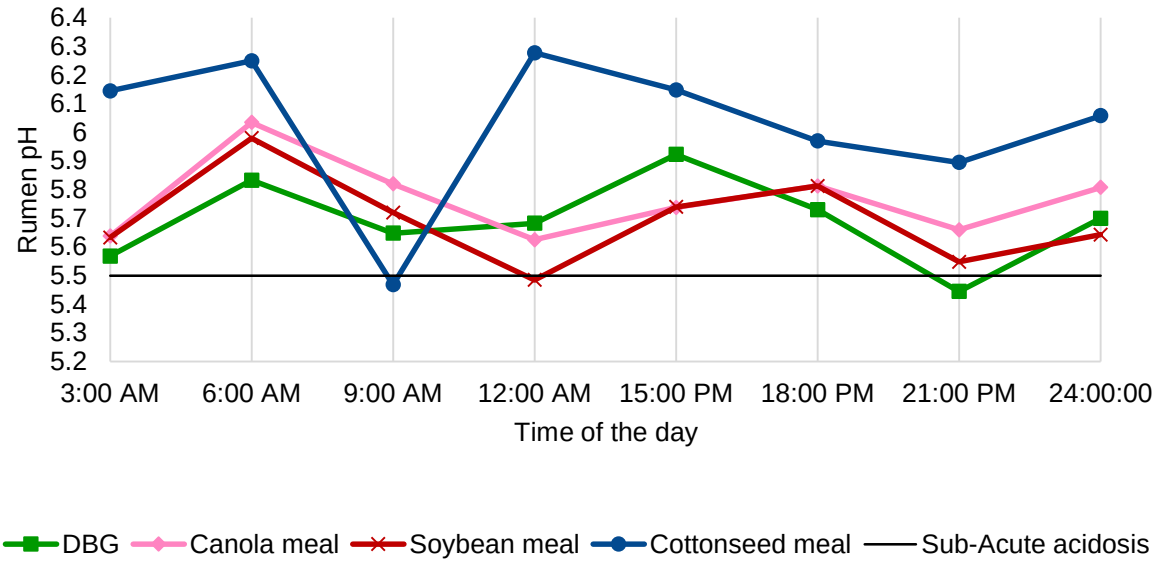


Figure 6.1 Fluctuation in ruminal pH over a 24-hour period in sheep fed different protein concentrates

Acidosis can be classified into one of two categories (González *et al.*, 2012), namely: acute (clinical) acidosis and sub-acute (sub-clinical) acidosis. Acute acidosis is a life-threatening condition which is a consequence of the overconsumption of readily fermentable carbohydrates, whereas an animal experiencing sub-acute acidosis rarely presents itself as physically ill, however reductions in performance and feed intake may occur (Freer &Dove, 2002; González *et al.*, 2012). A pH value ranging between 5.0 - 5.5 is considered as sub-acute acidosis whereas a pH value of below 5.0, nearing 4.5 or less, is regarded as acute acidosis (Nagaraja &Lechtenberg, 2007). The pH was, however, not recorded using an indwelling pH meter with probe (submersed in the rumen fluid), which logs pH data at minute-intervals. Thus, the exact period of time that an animal experienced sub-acute acidosis during this trial, cannot be accurately verified. Nonetheless, it can be presumed from Figure 6.1 above that although the pH values of the DBG, cottonseed- and soybean meal treatments did drop below 5.5 (sub-acute acidosis), this did not persist for an extended period of time. Consequently, the likelihood of negative effects of sub-acute acidosis were avoided.

The rumen pH of the cottonseed meal treatment sharply declined from 6:00am to 9:00am (Figure 6.1). This sudden drop can be attributed to a single wether which experienced sub-acute acidosis (pH of 5.11) at this specific time point, when consuming the cottonseed meal diet. The ruminal epithelium plays a vital role in the metabolism as well as absorption of nutrients and end products of microbial fermentation, thus damage to the ruminal papillae may result in reduced animal performance (Almeida *et al.*, 2018). From Figures 6.2 and 6.3, it is evident that the ruminal papillae of this cannulated sheep (P-1303) were compromised. The NRC (2007) stated that papillae should completely cover the lining of the rumen, with the size

and concentration of papillae varying due to conditions such as: stage of development, ruminal environment, dietary response and seasonal influences. Figure 6.4 shows the “healthy” rumen of a feedlot lamb, which was taken on slaughter day, included for comparative purposes of the ruminal epithelia. Furthermore, in the instance that ruminal papillae are damaged or where rumentitis is prevalent, the absorptive capability of the ruminal wall is altered, hindering the animals ability to maintain a balanced rumen pH (Nagaraja &Lechtenberg, 2007). Therefore, due to the compromised state of the ruminal epithelium of this cannulated sheep, the ability of this animal to regulate its pH was negatively affected.

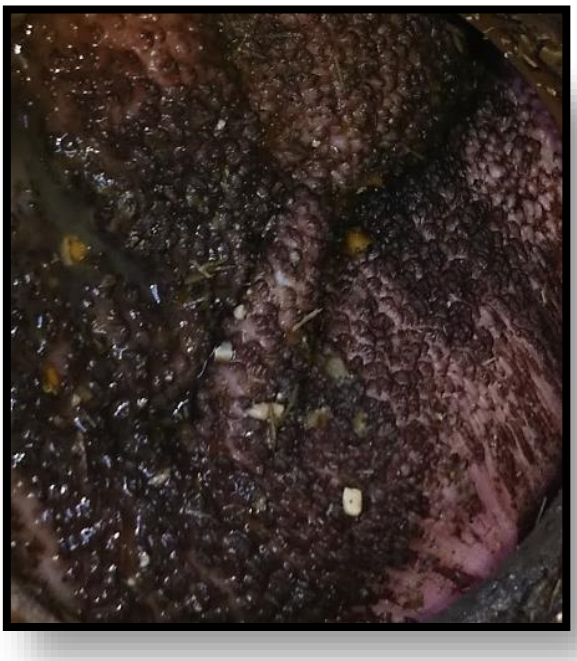


Figure 6.2 (Left) Photographic image of the cannulated sheep’s (P1303) ruminal epithelium
Figure 6.3 (Right) Magnified image of Figure 6.2, note damage of ruminal papillae

Figure 6.4 Photographic image of a feedlot lambs' ruminal epithelium

Rumen ammonia nitrogen concentration

A pivotal intermediate in the microbial degradation of protein as well as protein synthesis, is the ammonia nitrogen present in the rumen liquor (Freer *et al.*, 2007; McDonald *et al.*, 2011). When a diet is deficient in protein or when a protein source is resistant to ruminal degradation, a lower concentration of rumen NH_3-N will be present and ruminal microbial growth and MCP production will be stunted (Petit *et al.*, 1997). The opposite is therefore also true; if protein degradation in the rumen exceeds protein synthesis, rumen NH_3-N will accumulate. An excess of NH_3-N in the rumen can potentially be absorbed into the bloodstream, transported to the liver and subsequently transformed into urea. Some of this urea synthesised in the liver can be recycled back to the rumen; by means of direct absorption through the rumen wall or alternatively via the saliva, however, the majority is usually excreted in the urine and is hence wasted. Furthermore, this is an energy demanding process, thereby wasting energy that could have been utilized for production purposes (Coleman &Moore, 2003; McDonald *et al.*, 2011).

The range of the rumen NH_3-N concentration for the current study was 22.6 - 28.5 mg NH_3-N/ 100ml (at a urea inclusion of 1.23%), which falls within the range (8.5mg/100ml-30mg/100ml) for optimal production, as proposed by McDonald *et al.* (2011). In a similar trial, O'Reilly (2017) reported a rumen ammonia nitrogen range of 15.2 - 20.0 mg NH_3-N/100ml for sheep receiving high concentrate diets containing alternative levels of CMS, with the CP content of all diets being 16% with urea inclusion rates of 0.83%. Although the CP contents of

the diets from the current study was lower, an increase in dietary urea supplementation can nonetheless result in higher rumen NH_3-N concentrations (Alawa *et al.*, 1988). This is because bacterial urease ensures the rapid hydrolysis of urea in the rumen, therefore increasing the rumen ammonia nitrogen concentration (Freer *et al.*, 2007). This was demonstrated in a study by Alawa *et al.* (1988); cows fed fresh or dried brewers grain diets were supplemented with urea or with no urea. Higher rumen NH_3-N concentrations were reported for both fresh and dried brewers grain diets supplemented with urea, when compared to with those which were not supplemented with urea. For the DBG diet, Alawa *et al.* (1988) reported rumen NH_3-N concentrations of 25.9mg/100ml and 48.2mg/100ml for the un-supplemented and urea-supplemented diets, respectively. The rumen NH_3-N concentrations were higher than those reported in this study, as the CP content of experimental diets from the Alawa *et al.* (1988) study were considerably higher (DBG contained of 19.7% CP, *ad libitum* straw contained 3% CP).

No differences ($P>0.05$) were detected in the average ruminal NH_3-N concentration of sheep fed the four respective experimental diets. Nonetheless, elevated rumen NH_3-N concentrations can be indicative of excessive RDP concentrations which have not been synchronised with sufficient fermentable energy (Nagalakshmi *et al.*, 2003b). The highest average rumen NH_3-N concentration from the present study was measured in sheep receiving the DBG treatment. This was not anticipated for the DBG treatment, but rather for the canola meal treatment since DBG has been described in literature as a protein source characterised by a high RUP content (Westendorf &Wohlt, 2002) and was the protein source used in the current study with the second highest protein RUP content. In contrast, the canola meal had the lowest RUP content and Alawa *et al.* (1988) suggested that higher levels of RDP intake could subsequently lead to higher ruminal NH_3-N concentrations. However, from the degradability estimates in Section 6.3, the canola meal exhibited a greater CP disappearance from the *in sacco* bags than DBG. Therefore, it is possible that an imbalance occurred in the synchronisation of supply in fermentable energy and RDP in sheep fed the DBG diet. Nonetheless, Faccenda *et al.* (2018) stated that higher concentrations of ruminal NH_3-N may lead to elevated rates of protein synthesis by microbes.

The lowest mean rumen NH_3-N concentration for this study was reported for the cottonseed meal treatment. Lower NH_3-N values may imply that more protein has escaped rumen degradation and will be available for digestion in the small intestine (Nagalakshmi *et al.*, 2003b). The cottonseed meal was the protein concentrate used in the present study with the second highest RUP contribution according to literature. Nevertheless, from the *in sacco* results and RUP values as discussed, the lower NH_3-N concentrations observed in the current study can be justified.

The feed intake pattern and the specific diet which an animal is fed, will affect the ammonia concentrations in the rumen (Freer *et al.*, 2007). Animals were fed at 8:00 AM and 15:00 PM during the current study. As shown in Figure 6.5, rumen ammonia levels peaked in a similar fashion for the canola meal and soybean meal treatments; the highest concentrations were measured at 15:00 PM (at the afternoon feeding). The DBG treatment reached its peak rumen NH_3-N concentration at 9:00 AM (1-hour post feeding). Other researchers, however, reported that rumen NH_3-N concentrations peak between 2-4 hours post feeding (Alawa *et al.*, 1988; Batista *et al.*, 2016; Faccenda *et al.*, 2018). For the cottonseed meal treatment rumen NH_3-N concentrations peaked at 12:00 AM (4 hours post morning feeding). This corresponds with the findings of Nagalakshmi *et al.* (2003b), who also reported the highest NH_3-N concentrations 4 hours post-feeding for lambs fed diets supplemented with cottonseed-meal.

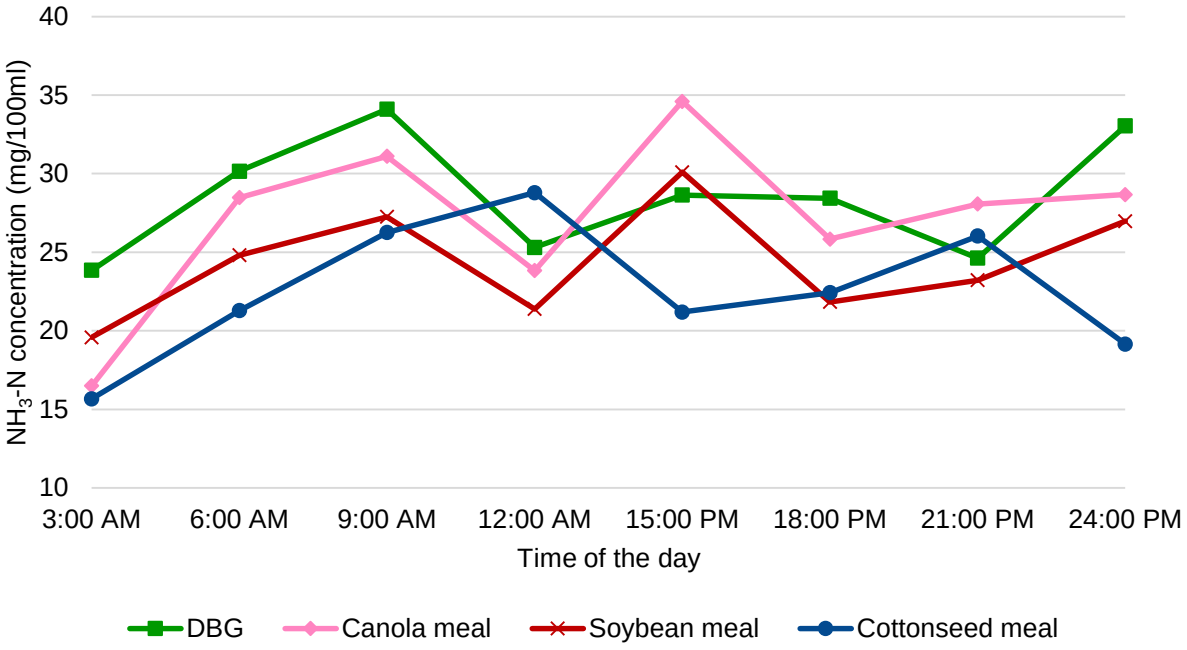


Figure 6.5 Variation in ruminal ammonia-nitrogen concentration over a 24-hour period in sheep fed different protein concentrates

The rumen NH_3-N concentration of the DBG treatment showed a tendency ($P<0.10$) to be higher than the cottonseed treatment at the 24:00 PM sampling time. Similarly, a tendency ($P<0.10$) of the DBG treatment to be higher than the canola- and cottonseed meal treatments, was furthermore detected at the 3:00AM sampling time. In the literature studied, no evidence could be found to explain the differences observed. The variation in feeding pattern between individual sheep most probably contributed to the variation observed.

Chapter 7

Conclusion

From the feedlot growth trial, the average total weight gained and ADG over the feedlot period was lower ($P<0.05$) for the canola meal treatment, in comparison to the other three experimental diets. It was assumed that due to the higher body weight of these animals at the start of the trial, their growth was less efficient, culminating in the aforementioned inferior performance. A higher ($P<0.05$) feed intake was realised for the cottonseed meal treatment, when compared to the other three experimental diets. Furthermore, a lower ($P<0.05$) feed intake was realised for the DBG treatment, when compared to the other three treatment diets. These results were in disagreement with the findings of the 4×4 Latin square design. The differences in feed intake observed amongst the two studies, were assumed to have been influenced by: previous feed exposure (age), weather conditions, inherent quality and nutrient attributes of the experimental diets. The FCR of the DBG diet was better ($P<0.05$) than that of the canola meal, soybean meal and cottonseed meal treatments respectively. Contrastingly, the FCR of the lambs consuming the canola meal treatment was poorer ($P<0.05$) than the other three experimental diets.

Regarding the carcass parameters of the feedlot lambs; both hot and cold carcass weights exhibited identical statistical trend with the lambs consuming the DBG treatment, producing lower ($P<0.05$) carcass weights than the canola- and cottonseed meal treatments, respectively. The differences in carcass weights were presumably influenced by the final live body weight of lambs prior to slaughter. The dressing percentage of the canola meal treatment was higher ($P<0.10$) than that of the cottonseed meal treatment for the current study. Carcass classification remained unaffected ($P>0.05$) by treatment.

From the 4×4 Latin square design, it was evident that wethers consumed higher ($P<0.05$) quantities of the DBG diet, when compared to the soybean meal diet. It was proposed that this was due to the higher limestone inclusion rates in the soybean meal diet. A tendency ($P<0.10$) was detected in the current study, whereby the apparent DM digestibility of the DBG was lower than that of the soybean meal diet. This was attributed to the inherent characteristics of the nutrient fractions present in DBG. Additionally, the apparent CP digestibility of the soybean meal diet, was higher ($P<0.05$) than that of the DBG and canola meal diets. The differences in CP digestibility were assumed to have been due to the differences observed in the feed intakes of these respective diets, which ultimately influenced

the passage rate of the feed. The apparent total tract starch digestibility and NDF digestibility remained unaffected (P>0.05) by treatment for the current study.

The DM disappearance of the DBG was lower (P<0.05) for the present study, when compared to the canola-, soybean- and cottonseed meal treatments respectively. No differences (P>0.05) were identified between the CP disappearance of the four different protein concentrates. The failure to detect significant differences in the afore-mentioned fraction was proposed to have been due to the high experimental error, as the microbial contamination of bag residues was not accounted for in the present study. With regard to the rumen fermentation parameters, a higher (P<0.05) rumen pH was realised on the cottonseed meal diet, when compared to the other three experimental diets. It was suggested that the differences observed could be credited to the alternative rates of microbial activity; whereby the differences in the RDP characteristics of the four different protein concentrates contributed to alternative rates of ruminal microbial fermentation. However, this could not be completely verified as the proportions and concentrations of the different VFA were not analysed in the present study. Differences (P>0.05) were not established between the average rumen NH_3-N produced by the four different protein concentrates.

Considering both results delivered from the 4×4 Latin square design and the feedlot growth study, it can be proposed that the protein quality of the four different protein concentrates have the potential to impact the performance, digestibility and some rumen fermentation parameters of feedlot lambs.

In conclusion, DBG, canola meal, soybean meal and cottonseed meal all have the potential to be included in lamb feedlot rations, resulting in acceptable levels of performance, without adversely affecting the intake, digestibility or rumen fermentation of sheep.

Chapter 8

Critical Evaluation

Prospective research

Several ruminant studies which were reviewed, investigated different inclusion rates of a number of protein sources (Öster *et al.*, 1977; Bovolenta *et al.*, 1998; Silva *et al.*, 2016). This specific trial merely investigated the effects of protein quality on the performance of feedlot lambs at a specific inclusion rate of the protein in order to yield iso-nitrogenous diets. However, the effects of these different sources on performance can be tested at different inclusion rates, under South African feedlot conditions. Therefore, it can be determined whether a higher inclusion rate of a specific protein source leads to increased production, which could still prove to be economically viable for feedlot operations.

It was evident from the research conducted by Bovolenta *et al.* (1998), that associative effects are possible when combining different protein sources; this could, as a result, serve as a point of research in the future.

Some lambs from the present study did not adapt in the commonly used 2-week adaptation period, therefore this period was extended. Consequently, there is a need to determine the length of the adaptation period for animals which are weaned from different nutritional backgrounds. For example, the length of the adaptation period could be determined for lambs receiving creep feed prior to weaning, versus those weaned solely from pasture with no supplementation of concentrates.

Despite the poor productive performance of the lambs consuming the canola meal treatment, it could still serve as an important feed component. This owing to the higher oil content of this specific oilcake meal, which could lead to reduced methane emissions, as suggested by He *et al.* (2013). Additionally, its amino acid profile has the potential to be beneficial in sheep production (Khalid *et al*., 2012; Canola Council of Canada, 2015). Therefore, further research needs to be conducted regarding the use of canola meal as a feedstuff.

Experimental diets

It should be emphasised that care must be exercised during the production of feed pellets. Specific reference is made to the temperatures employed during the pelleting process and the structure of the final product. This is because several nutrients and amino acids (Dale,

1996) are heat-sensitive and can therefore be rendered unavailable, particularly once Maillard reactions have taken place (Thomas *et al.*, 1998). Konishi *et al.* (1999) stated that phosphorus may also be rendered unavailable during extreme heat exposure, specifically in oilseed meals. The majority of phosphorus in oilseed meals are present in the phytate-form (Konishi *et al.*, 1999). Phytates can be degraded in the rumen by micro-organisms, however, oilseed meals exposed to high temperatures during processing can subsequently have reduced phytate degradation abilities. This owing to the reduced solubility of protein-phytate complexes (Konishi *et al.*, 1999) in the rumen. Phytate, therefore, escapes the rumen undegraded and is consequently inadequately absorbed in the small intestine. Moreover, the intakes of the experimental diets which differed between the two different studies could have transpired due to the taste and/ or poor pellet quality.

4×4 Latin square design

The number of bags incubated during the *in sacco* procedure could have been increased to three or four bags per wether, per treatment. In addition, the MC contamination of the bag residues could additionally, have been accounted for by using methods employed by Millis *et al.* (2007) or Paz *et al*. (2014). This may, consequentially, have produced more accurate results with a reduced experimental error.

Numerous forgoing studies subjected sheep from their growth/feedlot experiments, to their metabolic studies (Khan *et al.*, 1997; Petit *et al.*, 1997; Bovolenta *et al.*, 1998; Nagalakshmi *et al.*, 2003b; Ward *et al.*, 2008). This may potentially have resulted in the collection of more accurate results, as these animals represent those that were exposed to the feedlot environment. In addition, the potential loss of digesta from un-cannulated animals is eliminated, thus more accurate digestibility estimates could be obtained. Moreover, the age of the cannulated wethers used in the present study, may possibly have swayed them in preferring a specific diet, hence altering the digestibility estimates.

Similarly, younger cannulated sheep could suggestively be used in research trials; thus, the likely occurrence of negative consequences, such as ruminal papillae damage influencing results, could be avoided.

The *in sacco* study could be performed prior to the rumen fluid collections in future. The *in sacco* study was conducted after the 18:00 collection time on day four in the present study and the cannulae of sheep were not opened for the duration of the incubation time. However, owing to the anaerobic microbial population present in the rumen (McDonald *et al.*, 2011), the opening and closing of the cannulae in order to retrieve ruminal fluid prior to the degradability study, resulted in the ruminal microbes being exposed to oxygen. This could have adversely

impacted the microbial population, thus subsequently influencing the degradability estimates of the feedstuffs. In addition, the two meals provided on a daily basis, can be separated by an 8-hour interval, as suggested by Madsen & Hvelplund (1994).

References

AFRC, 1993. Energy and Protein Requirements of Ruminants: An Advisory Manual. CAB International, Wallingford, UK.

Agenbag, A., 2015. Canola Production and Utilisation: An Overview: Agronomy. Oilseeds Focus. 1**,** 6-7.

Alawa, J., Fishwick, G. & Hemingway, R., 1988. Fresh and Dried Brewers' Grains as Protein Supplements to Barley Straw Diets Given to Pregnant Beef Cows. Ani. Feed Sci. Tech. 19**,** 33-41.

Almeida, M. T., Ezequiel, J. M., Paschoaloto, J. R., Perez, H. L., Carvalho, V. B., Castro Filho, E. S. & van Cleef, E. H., 2018. Rumen and Liver Measurements of Lambs Fed with High Inclusions of Crude Glycerin in Adaptation and Finishing Period of Feedlot. Small Ruminant Res. 167**,** 1-5.

AOAC, 2000. Official Method of Analysis (17th ed.). Assosciation of Official Analystical Chemists, Inc.,Maryland, USA.

Armentano, L., Herrington, T., Polan, C., Moe, A., Herbein, J. & Umstadt, P., 1986. Ruminal Degradation of Dried Brewers Grains, Wet Brewers Grains, and Soybean Meal. J. Dairy Sci. 69**,** 2124-2133.

Aufrere, J. & Michalet-Doreau, B., 1988. Comparison of Methods for Predicting Digestibility of Feeds. Anim. Feed Sci. Tech. 20**,** 203-218.

Batista, E., Detmann, E., Titgemeyer, E. C., Valadares Filho, S., Valadares, R., Prates, L., Rennó, L. & Paulino, M., 2016. Effects of Varying Ruminally Undegradable Protein Supplementation on Forage Digestion, Nitrogen Metabolism, and Urea Kinetics in Nellore Cattle Fed Low-Quality Tropical Forage. J. Anim. Sci. 94**,** 201-216.

Beauchemin, K., McClelland, L., Kozub, G. & Jones, S., 1995. Effects of Crude Protein Content, Protein Degradability and Energy Concentration of the Diet on Growth and Carcass Characteristics of Market Lambs Fed High Concentrate Diets. Can. J. Anim. Sci. 75**,** 387-395.

Bell, J., 1993. Factors Affecting the Nutritional Value of Canola Meal: A Review. Can. J. Anim. Sci. 73**,** 689-697.

Boisen, S., Hvelplund, T. & Weisbjerg, M. R., 2000. Ideal Amino Acid Profiles as a Basis for Feed Protein Evaluation. Livest. Prod. Sci. 64**,** 239-251.

Bovolenta, S., Piasentier, E., Peresson, C. & Malossini, F., 1998. The Utilization of Diets Containing Increasing Levels of Dried Brewers' Grains by Growing Lambs. Anim. Sci. 66**,** 689-695.

Bowen, M., Ryan, M., Jordan, D., Beretta, V., Kirby, R., Stockman, C., McIntyre, B. & Rowe, J., 2006. Improving Sheep Feedlot Management. Int. J. Sheep Wool Sci. 54**,** 27-34.

Brand, T., van der Westhuizen, E., van der Merwe, D. & Hoffman, L., 2017. Effect of Days in Feedlot on Growth Performance and Carcass Characteristics of Merino, South African Mutton Merino and Dorper Lambs. S. Afr. J. Anim. Sci. 47**,** 26-33.

Brand, T., Van Der Westhuizen, E., van Der Merwe, D. & Hoffman, L., 2018. Analysis of Carcass Characteristics and Fat Deposition of Merino, South African Mutton Merino and Dorper Lambs Housed in a Feedlot. S. Afr. J. Anim. Sci. 48**,** 477-488.

Broderick, G. & Kang, J., 1980. Automated Simultaneous Determination of Ammonia and Total Amino Acids in Ruminal Fluid and in Vitro Media1. J. Dairy Sci. 63**,** 64-75.

Burger, A., Hoffman, L. C., Cloete, J., Muller, M. & Cloete, S., 2013. Carcass Composition of Namaqua Afrikaner, Dorper and Sa Mutton Merino Ram Lambs Reared under Extensive Conditions. S. Afr. J. Anim. Sci. 43**,** 27-32.

Busanello, M., Velho, J., Alessio, D., Haygert-Velho, I., Tambara, A. & Neto, A. T., 2017. In Situ Ruminal Degradability of Protein Feeds with Distinct Physical Forms: A Meta-Analysis. S. Afr. J. Anim. Sci. 47**,** 91-95.

Canola Council of Canada. 2015. Canola Meal Feeding Guide. Available: www.canolacouncil.org (Accessed 30 March 2018).

Casey, N. & Webb, E., 1995. Influence of Dietary Energy Levels and Form of Diet on Composition of Fatty Acids in Subcutaneous Adipose Tissue of Wethers. Small Ruminant Res. 18**,** 125-132.

Cloete, J., Hoffman, L. & Cloete, S., 2012. A Comparison between Slaughter Traits and Meat Quality of Various Sheep Breeds: Wool, Dual-Purpose and Mutton. Meat Sci. 91**,** 318-324.

Coleman, S. W. & Moore, J. E., 2003. Feed Quality and Animal Performance. Field Crop. Res. 84**,** 17-29.

Cornelius, P., 2017. Mutton Outlook Report March 2017 [Online]. AMT (Pty) Ltd. Available: http://www.redmeatsa.co.za/wp-content/uploads/2017/03/AMT-Mutton-Outlook-March-2017.pdf [Accessed 30 March 2018].

Cruywagen, C., 2006. A Method to Facilitate Retrieval of Polyester Bags Used in in Sacco Trials in Ruminants. J. Dairy Sci. 89**,** 1028-1030.

Cruywagen, C., Lategan, E. & Hoffman, L., 2003. The Effect of Rumen Inert Fat Supplementation and Protein Degradability in Starter and Finishing Diets on Veal Calf Performance. S. Afr. J. Anim. Sci. 33**,** 257-265.

Dale, N., 1996. Variation in Feed Ingredient Quality: Oilseed Meals. Anim. Feed Sci. Tech. 59, 129-135.

Du Toit, C., 2006. The Effect of Type and Level of Carbohydrate Supplementation on Intake and Digestibility of Atriplex Nummularia Cv. De Kock Fed to Sheep. MSc(Agric): Animal Science, dissertation, Dept. of Animal and Wildlife Sciences, University of Pretoria, South Africa.

Engels, E. & Van der Merwe, F., 1967. Application of an in Vitro Technique to South African Forages with Special Reference to the Effect to Certain Factors on the Results. S. Afr. J. Anim. Sci. 10, 983-995.

Erasmus, L., Botha, P., Cruywagen, C. & Meissner, H., 1994. Amino Acid Profile and Intestinal Digestibility in Dairy Cows of Rumen-Undegradable Protein from Various Feedstuffs. J. Dairy Sci. 77, 541-551.

Erasmus, L., Prinsloo, J. & Meissner, H., 1988. The Establishment of a Protein Degradability Data Base for Dairy Cattle Using the Nylon Bag Technique. 1. Protein Sources. S. Afr. J. Anim. Sci. 18, 23-29.

Erickson, G.E., Watson, A.K., Mac Donald, J.C. & Klopsfontein, T.J., 2016. Protein nutrition evaluation and application to growing and finishing cattle. Proc. Florida Rum. Nutr. Conf., Gainesville, KL.

Faccenda, A., Zambom, M., Avila, A., Fernandes, T., Stum, M., Garcias, J., Tinini, R. & Dias, A., 2018. Dried Brewers' Grain as a Replacement for Soybean Meal on Nutrient Digestibility and Rumen Parameters of Cattle. S. Afr. J. Anim. Sci. 48.

Freer, M. & Dove, H. 2002. Sheep Nutrition. CABI,CSIRO Publishing, Collingwood, Australia.

Freer, M., Dove, H. & Nolan, J. 2007. Nutrient Requirements of Domesticated Ruminants. CSIRO publishing.

Gibb, D., Hao, X. & McAllister, T., 2008. Effect of Dried Distillers' Grains from Wheat on Diet Digestibility and Performance of Feedlot Cattle. Can. J. Anim. Sci. 88, 659-665.

Giron, H. C., 1973. Atomic Absorption Newsletter 12, 28. Perkin Elmer Atomic Spectrophotometer.

Gomez, K. A., Gomez, K. A. & Gomez, A. A. ,1984. Statistical Procedures for Agricultural Research. John Wiley & Sons.

González, L., Manteca, X., Calsamiglia, S., Schwartzkopf-Genswein, K. & Ferret, A., 2012. Ruminal Acidosis in Feedlot Cattle: Interplay between Feed Ingredients, Rumen Function and Feeding Behavior (a Review). Anim. Feed Sci. Tech. 172, 66-79.

Good, A. C., 2018. Evaluation of Canola Meal Versus Soybean Meal as a Protein Supplement for Beef Cattle: Effects on Growth Performance, Carcass Characteristics, Rumen Fermentation, and Nutrient Digestion. MSc Animal Science , dissertation, Dept. of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada.

He, M. L., Gibb, D., McKinnon, J. J. & McAllister, T. A., 2013. Effect of High Dietary Levels of Canola Meal on Growth Performance, Carcass Quality and Meat Fatty Acid Profiles of Feedlot Cattle. Can. J. Anim. Sci. 93**,** 269-280.

Herrman, T. J., 2001. Sampling: Procedures for Feed [Online]. Kansas State University. Available: https://www.bookstore.ksre.ksu.edu/pubs/mf2036.pdf [Accessed 25 February 2019].

Huntington, G., Harmon, D. & Richards, C., 2006. Sites, Rates, and Limits of Starch Digestion and Glucose Metabolism in Growing Cattle. J. Anim. Sci. 84**,** 14-24.

Kandylis, K., Nikokyris, P. N. & Deligiannis, K., 1999. Performance of Growing–Fattening Lambs Fed Diets Containing Different Proportions of Cotton Seed Meal.J. Sci. Food Agr. 79**,** 1613-1619.

Kaps, M. & Lamberson, W. R., 2009. Biostatistics for Animal Science (2nd ed). CABI.

Kerley, G. I. H., Behrens, K. G., Carruthers, J., Diemont, M., du Plessis, J., Minnie, L., Richardson, P. R. K., Somers, M. J., Tambling, C. J., Turpie, J., van Niekerk, H. N. & Balfour, D., 2017. Livestock Predation in South Africa: The Need for and Value of a Scientific Assessment. S. Afr. J. Anim. Sci. 113**,** 1-3.

Khalid, M., Sarwar, M., Rehman, A., Shahzad, M. & Mukhtar, N., 2012. Effect of Dietary Protein Sources on Lamb's Performance: A Review. Iranian J. Appl. Anim. Sci. 2, 111-120.

Khan, A., Azim, A., Nadeem, M. & Khan, M., 1997. Effect of Growing Fattening Diets on the Growth Performance of Intensified Afghani Lambs. Small Ruminant Res. 25**,** 39-42.

Konishi, C., Matsui, T., Park, W., Yano, H. & Yano, F., 1999. Heat Treatment of Soybean Meal and Rapeseed Meal Suppresses Rumen Degradation of Phytate Phosphorus in Sheep. Anim. Feed Sci. Tech. 80**,** 115-122.

Lardy, G. P. & Anderson, V., 2002. Canola and Sunflower Meal in Beef Cattle Diets. Vet. Clin. N. Am. 18**,** 327-338.

Madsen, J. & Hvelplund, T., 1994. Prediction of in Situ Protein Degradability in the Rumen. Results of a European Ringtest. Livest. Prod. Sci. 39**,** 201-212.

McCarthy, A. L., O'Callaghan, Y. C., Piggott, C. O., FitzGerald, R. J. & O'Brien, N. M., 2013. Brewers' Spent Grain; Bioactivity of Phenolic Component, Its Role in Animal Nutrition and Potential for Incorporation in Functional Foods: A Review. P. Nutr. Soc. 72**,** 117-125.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A. & Wilkinson, R. G. 2011. Animal Nutrition 7th ed. Pearson United Kingdom, Harlow, England.

McNiven, M. A., Prestløkken, E., Mydland, L. & Mitchell, A., 2002. Laboratory Procedure to Determine Protein Digestibility of Heat-Treated Feedstuffs for Dairy Cattle. Anim. Feed Sci. Tech. 96**,** 1-13.

Mentz, A., Van Niekerk, W. A., Hassen, A., Coertze, R. J. & Gemeda, B. S., 2015. Effect of Diets Differing in Rumen Soluble Nitrogen on Utilization of Poor-Quality Roughage by Sheep. S. Afr. J. Anim. Sci. 45**,** 528-537.

Merchen, N. R. & Titgemeyer, E. C., 1992. Manipulation of Amino Acid Supply to the Growing Ruminant. J. Anim. Sci. 70**,** 3238-3247.

Michalet-Doreau, B. & Ould-Bah, M., 1992. In Vitro and in Sacco Methods for the Estimation of Dietary Nitrogen Degradability in the Rumen: A Review. Anim. Feed Sci. Tech. 40**,** 57-86.

Milis, C., Liamadis, D., Karatzias, C. & Abas, Z., 2007. Nitrogen in Vivo Digestibility and in Situ Degradability Data for Estimation of Lower Tract N Digestibility with or without Correction for Microbial Contamination. Small Ruminant Res. 71**,** 205-214.

Mukherjee, R., Chakraborty, R. & Dutta, A., 2016. Role of Fermentation in Improving Nutritional Quality of Soybean Meal—a Review. Asian Austral. J. Anim. 29**,** 1523.

Mynhardt, H., Van Ryssen, J. & Coertze, R., 2006. The Effect of the Heat Processing of Soybean Seed on the Metabolism of Its Selenium in Lambs. Anim. Feed Sci. Tech. 128**,** 122-134.

Nagalakshmi, D., Sastry, V. & Agrawal, D., 2003a. Relative Performance of Fattening Lambs on Raw and Processed Cottonseed Meal Incorporated Diets. Asian Austral. J. Anim. 16**,** 29-35.

Nagalakshmi, D., Sastry, V. & Pawde, A., 2003b. Rumen Fermentation Patterns and Nutrient Digestion in Lambs Fed Cottonseed Meal Supplemental Diets. Anim. Feed Sci. Tech. 103**,** 1-14.

Nagaraja, T. & Lechtenberg, K. F., 2007. Acidosis in Feedlot Cattle. Vet. Clin. N. Am. 23**,** 333-350.

Nair, J., Penner, G., Yu, P., Lardner, H., McAllister, T., Damiran, D. & McKinnon, J., 2016. Evaluation of Canola Meal Derived from Brassica Juncea and Brassica Napus on Rumen Fermentation and Nutrient Digestibility by Feedlot Heifers Fed Finishing Diets. Can. J. Anim. Sci. 96**,** 342-353.

Nandi, S., De, U. K. & Chowdhury, S., 2011. Current Status of Contagious Ecthyma or Orf Disease in Goat and Sheep—a Global Perspective. Small Ruminant Res. 96**,** 73-82.

Nolan, J. V., Hegarty, R., Hegarty, J., Godwin, I. & Woodgate, R., 2010. Effects of Dietary Nitrate on Fermentation, Methane Production and Digesta Kinetics in Sheep. Anim. Prod. Sci. 50**,** 801-806.

Nolte, J. v. E. & Ferreira, A., 2004. Energy and Nitrogen Retention of Merino and Dohne Merino Lambs Receiving a Feedlot Diet. S. Afr. J. Anim. Sci. 34**,** 77-79.

NRC, 1985a . Nutrient Requirements of Sheep (6th Rev. Edition). National Academy of Sciences, Washington, D.C., USA. (cited by Beauchemin *et al.*,1995)

NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. The National Academies Press, Washington D.C., USA.

O'Reilly, K. 2017. Effect of Condensed Molasses Solubles on Intake Growth Performance Digestibility and Certain Rumen Parameters of Sheep. MSc(Agric): Animal Science ,dissertation, Dept. of Animal and Wildlife Sciences, University of Pretoria, South Africa.

Ørskov, E., Hovell, F. d. B. & Mould, F., 1980. The Use of Nylon Bag Technique for the Evaluation of Feedstuffs. Trop. Anim. Prod. 5**,** 195-213.

Ørskov, E. & McDonald, I., 1979. The Estimation of Protein Degradability in the Rumen from Incubation Measurements Weighted According to Rate of Passage. J. Agr. Sci. 92**,** 499-503.

Öster, A., Thomke, S. & Gyllang, H., 1977. A Note on the Use of Brewers' Dried Grains as a Protein Feedstuff for Cattle. Anim. Sci. 24**,** 279-282.

Osuji, P.O., Nsahlai, I.V. & Khalili, H., 1993. Feed Evaluation ILCA Manual. International Livestock Centre for Africa. Addis Ababa, Ethiopia.

Owens, F. N., Gill, D. R., Secrist, D. S. & Coleman, S., 1995. Review of Some Aspects of Growth and Development of Feedlot Cattle. J. Anim. Sci. 73**,** 3152-3172.

Paz, H., Klopfenstein, T., Hostetler, D., Fernando, S. C., Castillo-Lopez, E. & Kononoff, P., 2014. Ruminal Degradation and Intestinal Digestibility of Protein and Amino Acids in High-Protein Feedstuffs Commonly Used in Dairy Diets. J. Dairy Sci. 97**,** 6485-6498.

Petit, H. V., Rioux, R., D'oliveira, P. & Prado, I. D., 1997. Performance of Growing Lambs Fed Grass Silage with Raw or Extruded Soybean or Canola Seeds. Can. J. Anim. Sci. 77**,** 455-463.

Ponnampalam, E. N., Egan, A. R., Sinclair, A. J. & Leury, B. J., 2005. Feed Intake, Growth, Plasma Glucose and Urea Nitrogen Concentration, and Carcass Traits of Lambs Fed Isoenergetic Amounts of Canola Meal, Soybean Meal, and Fish Meal with Forage Based Diet. Small Ruminant Res*.* 58**,** 245-252.

Preston, R., 2016. 2016 Feed Composition Table. Beef Magazine*.* 51**,** 14-29. Available: https://www.beefmagazine.com/sites/beefmagazine.com/files/2016-feed-composition-tables-beef-magazine.pdf [Accessed 8 July 2019].

Ramachandran, S., Singh, S. K., Larroche, C., Soccol, C. R. & Pandey, A., 2007. Oil Cakes and Their Biotechnological Applications–a Review. Bioresource Technol*.* 98**,** 2000-2009.

Robertson, J. B. & Van Soest, P. J., 1981. The Analysis of Dietary Fibre in Food. Dekker, New York. ADSRI-JAN 1988.

Robinson, P., Givens, D. & Getachew, G., 2004. Evaluation of Nrc, Uc Davis and Adas Approaches to Estimate the Metabolizable Energy Values of Feeds at Maintenance Energy Intake from Equations Utilizing Chemical Assays and in Vitro Determinations. Anim. Feed Sci. Tech*.* 114**,** 75-90.

Rogers, J., Conrad, H., Dehority, B. & Grubb, J., 1986. Microbial Numbers, Rumen Fermentation, and Nitrogen Utilization of Steers Fed Wet or Dried Brewers' Grains. J. Dairy Sci*.* 69**,** 745-753.

Sami, A., Schuster, M. & Schwarz, F., 2010. Performance, Carcass Characteristics and Chemical Composition of Beef Affected by Lupine Seed, Rapeseed Meal and Soybean Meal. J. Anim. Physiol. An. N *.* 94**,** 465-473.

Samuels, M. L. 1989. Statistics for the Life Sciences. Collier MacMillan Publishers,London, England.

Santos, F. A. P., Santos, J., Theurer, C. & Huber, J. T., 1998. Effects of Rumen-Undegradable Protein on Dairy Cow Performance: A 12-Year Literature Review. J. Dairy Sci*.* 81**,** 3182-3213.

SAPA, 2018. South African Poultry Assosciation Feed Ingredient Report 2018 [online]. Available: http://www.sapoultry.co.za/ [Accessed 3 October 2019].

SAS 2019. Statistical Analysis Systems User's Guide: Statistics Version 9.3. SAS Institute Inc.,Cary, North Carolina, USA.

Savage, D., Ferguson, D., Fisher, A., Hinch, G., Mayer, D., Duflou, E., Lea, J., Baillie, N. & Raue, M., 2008. Preweaning Feed Exposure and Different Feed Delivery Systems to Enhance Feed Acceptance of Sheep. Aust. J. of Exp. Agr*.* 48**,** 1040-1043.

Savell, J.W., Mueller, S.L. & Baird, B.E., 2005. The Chilling of Carcasses. Meat Sci. 70, 449-459.

Schroeder, G., Erasmus, L., Leeuw, K. & Meissner, H., 1996. The Use of Acid Detergent Insoluble Nitrog to Predict Digestibility of Rumen Undergradable Protein of Heat Processed Plant Proteins'. S. Afr. J. Anim. Sci. 26.

Schwab, C. G., Tylutki, T., Ordway, R., Sheaffer, C. & Stern, M., 2003. Characterization of Proteins in Feeds. J. Dairy Sci. 86**,** 88-103.

Shaver, R. D., 2013. By-Product Feedstuffs in Dairy Cattle Diets in the Upper Midwest. Forage Resources & Information. UW Extension.

Sheridan, R., Ferreira, A. & Hoffman, L., 2003. Production Efficiency of South African Mutton Merino Lambs and Boer Goat Kids Receiving Either a Low or a High Energy Feedlot Diet. Small Ruminant Res. 50**,** 75-82.

Silva, R. V. M. M., de Carvalho, G. G. P., Pires, A. J. V., Pereira, M. L. A., Pereira, L., Campos, F. S., Perazzo, A. F., de Oliveira Nascimento, C., Santos, S. A. & Tosto, M. S. L., 2016. Cottonseed Cake in Substitution of Soybean Meal in Diets for Finishing Lambs. Small Ruminant Res. 137**,** 183-188.

Souza, C., Oliveira, R., Voltolini, T., Menezes, D., dos Santos, N., Barbosa, A., Silva, T., Pereira, E. & Bezerra, L., 2018. Lambs Fed Cassava Silage with Added Tamarind Residue: Silage Quality, Intake, Digestibility, Nitrogen Balance, Growth Performance and Carcass Quality. Anim. Feed Sci. Tech. 235**,** 50-59.

Stanton, T. L., LeValley, S. B. & Swanson, V. B., 2006. Lamb Feedlot Nutrition. Fact sheet 1.613. Colorado State University.

Stern, M. D., Bach, A. & Calsamiglia, S., 2006. New Concepts in Protein Nutrition of Ruminants. 21st Annual Southwest Nutrition & Management Conference. 45-62.

Thomas, M., Van Vliet, T. & Van der Poel, A., 1998. Physical Quality of Pelleted Animal Feed 3. Contribution of Feedstuff Components. Anim. Feed Sci. Tech. 70**,** 59-78.

Tilley, J. M. A. & Terry, R. A., 1963. A Two Stage Technique for the in Vitro Digestion of Forage Crops. J. Brit. Grassland Society . 18**,** 104.

Van Niekerk, W. A. & Hassen, A., 2009. Qualitative Evaluation of Four Subtropical Grasses as Standing Hay: Diet Selection, Rumen Fermentation and Partial Digestibility by Sheep. Afr. J. Range For. Sci. 26**,** 69-74.

Van Niekerk, W. A., Taute, A. & Coertze, R. J., 2002. An Evaluation of Nitrogen Fertilised Panicum Maximum Cv. Gatton at Different Stages of Maturity During Autumn: 2. Diet Selection, Intake, Rumen Fermentation and Partial Digestion by Sheep. S. Afr. J. Anim. Sci. 32.

Vasconcelos, J. & Galyean, M., 2007. Nutritional Recommendations of Feedlot Consulting Nutritionists: The 2007 Texas Tech University Survey. J. Anim. Sci. 85**,** 2772-2781.

Van Saun, R.J., 2006. Forage Testing: How to Translate Numbers into Actions. Proc. 2006 Mid-Atlantic States Conf. Bovine Pract.

Wanapat, M., Foiklang, S., Rowlinson, P. & Pilajun, R., 2012. Effect of Carbohydrate Sources and Cotton Seed Meal in the Concentrate: Ii. Feed Intake, Nutrient Digestibility, Rumen Fermentation and Microbial Protein Synthesis in Beef Cattle. Trop. Anim. Health Pro. 44**,** 35-42.

Ward, A., Tawila, G., Sawsan, M., Gad, M. & El-Muniary, M., 2008. Improving the Nutritive Value of Cottonseed Meal by Adding Iron on Growing Lambs Diets. World J. Agric. Sci. 4**,** 533-537.

Webb, E. C. 1994. Synthesis of Long Chain Fatty Acids in Ruminants and Their Effects on Meat Quality. PhD thesis (Animal Science), University of Pretoria, Pretoria, South Africa.

Webb, E. C., 2015. Description of Carcass Classification Goals and the Current Situation in South Africa. S. Afr. J. Anim. Sci. 45**,** 229-233.

Webb, E. C., Allen, J. & Morris, S., 2018. Effects of Non-Steroidal Growth Implant and Dietary Zilpaterol Hydrochloride on Growth and Carcass Characteristics of Feedlot Lambs. S. Afr. J. Anim. Sci. 48**,** 601-608.

Webb, E. C. & Erasmus, L. J., 2013. The Effect of Production System and Management Practices on the Quality of Meat Products from Ruminant Livestock. S. Afr. J. Anim. Sci. 43**,** 413-423.

Weisbjerg, M. R., Hvelplund, T., Hellberg, S., Olsson, S. & Sanne, S., 1996. Effective Rumen Degradability and Intestinal Digestibility of Individual Amino Acids in Different Concentrates Determined in Situ. Anim. Feed Sci. Tech. 62**,** 179-188.

Westendorf, M. L. & Wohlt, J. E., 2002. Brewing by-Products: Their Use as Animal Feeds. Vet. Clin. N. Am. 18**,** 233-252.

Wiese, S., White, C., Masters, D., Milton, J. & Davidson, R., 2003. Growth and Carcass Characteristics of Prime Lambs Fed Diets Containing Urea, Lupins or Canola Meal as a Crude Protein Source. Aust. J. Exp. Agr. 43**,** 1193-1197.

Zagorakis, K., Liamadis, D., Milis, C., Dotas, V. & Dotas, D., 2015. Nutrient Digestibility and in Situ Degradability of Alternatives to Soybean Meal Protein Sources for Sheep. Small Ruminant Res. 124**,** 38-44.

Zinn, R., Montano, M., Alvarez, E. & Shen, Y., 1997. Feeding Value of Cottonseed Meal for Feedlot Cattle. J. Anim. Sci. 75**,** 2317-2322.

Zinn, R.A., 2014. Amino Acid Nutrition of Feedlot Calves. Proc. Southwest Nutr. Mgmt. Conf.