An *in vitro* comparison of plant-based protein sources and their effects on feedlot lamb growth parameters

By

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Thesis submitted in partial fulfilment of the requirements for the degree Master of Science Animal Sciences in the Faculty of AgriScience at Stellenbosch University

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December 2022

DECLARATION

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ABSTRACT

Title:	An *in vitro* comparison of plant-based protein sources and their effects on feedlot lamb growth.
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Protein supplements are a very costly component of animal feed and the importance of allocating high-quality protein sources that is economically viable, consistent, and sustainable is important. By feeding human-inedible feed to ruminant animals to produce human-edible animal proteins will advance sustainability. An example of such an alternative high in protein feed source, is distiller's dried grains with solubles (DDGS). It is also a potentially low-cost protein source compared to oil seed meals. This study investigated the nutritional potential of DDGS to replace soybean meal in comparison with other protein raw materials, dried brewer grains (DBG), cottonseed meal (CSM), and the effect of the least cost optimal formulated diet with these protein sources in growing lamb diets.

In the first study (Chapter 3) the extent and rate of ruminal protein degradation of the different protein sources was determined against dried DDGS for ruminants. Five different protein samples, soybean meal, cottonseed meal, dried brewers grain, canola meal and DDGS were incubated for 0, 2, 4, 8, 16, 24, and 48 h. The effective degradability was calculated with an hourly disappearance rate of 5% and 8% per hour. The observed rumen undegradable protein content (k_p = 0.08/h) was the lowest for soybean meal (47%) followed by canola meal (49%), distillers' grains and solubles (50%), cottonseed meal (64%) and then dried brewers grain (68%). Non-significant differences were observed in the ruminal degradation rates between soybean meal and DDGS.

The second study (Chapter 4) investigated how the different protein treatment diets affected growth parameters, carcass characteristics and profitability of feedlot lambs. The treatment diets were formulated to be iso-nitrogenous, iso-energetic and contained one of five different protein sources, soybean meal, DDGS, cottonseed meal, dried brewers grain, and the least cost optimal formulation.

Thirty-five Dohne Merino wethers were allocated to treatments in a completely randomized design, with five treatments and seven replicates. No differences were observed in final body weight and feed efficiency as a result of the dietary treatment. Carcass weights also did not differ ($P > 0.05$). In conclusion, this trial determined that DDGS could economically replace soybean meal as a feed source to use in feedlot diets, and could be a valuable cost effective feed source to farmers and feed manufacturers.

OPSOMMING

Titel:	*In vitro* vergelyking van plantgebasseerde proteïnbronne and die effek daarvan op voerkraallamafronding.
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Graad:	MSc Agric

Proteïenaanvulling is 'n baie duur komponent van veerantsoene en die belangrikheid daarvan om hoë gehalte proteïenbronne te vind wat ekonomies, konsekwent en volhoubaar is, is van belang. Deur voerbronne wat nie deur mense benut kan word nie aan herkouerdiere te verskaf en in menslik benutbare diereproteïen om te skakel, sal volhoubaarheid bevorder. 'n Voorbeeld van so 'n alternatiewe voerbron, wat hoog is in proteïen, is gedistilleerde gedroogde grane met oplosbare stowwe (DDGS). Dit is ook 'n potensieel laekoste-proteïenbron in vergelyking met oliekoekproteïenbronne. Hierdie studie het die voedingspotensiaal van DDGS om sojaboonoliekoekmeel te vervang ondersoek in vergelyking met ander proteïenbevattende grondstowwe, gedroogde brouersgraan, katoensaadoliekoekmeel, en die goedkoopste optimale geformuleerde dieet (mengsel van genoemde proteïenbronne) in lamafrondrantsoene.

In die eerste studie (Hoofstuk 3) is die mate en tempo van ruminale proteïenafbraak van die verskillende proteïenbronne teen DDGS vir herkouers bepaal. Vyf verskillende proteïengrondstowwe insluitend sojaboonoliekoekmeel, katoensaadoliekoekmeel, gedroogde brouersgraan, kanolaoliekoekmeel en DDGS is vir 0, 2, 4, 8, 16, 24 en 48 h *in vitro* geïnkubeer. Die effektiewe afbreekbaarheid is bereken met 'n uurlikse verdwyntempo van 5% en 8% per uur. Die waargenome rumen-onafbreekbare proteïeninhoud (k_p = 0.08/h) was die laagste vir sojaoliekoekmeel (47%), gevolg deur kanolaoliekoekmeel (49%), DDGS (50%), katoensaadoliekoekmeel (64%) en dan gedroogde brouersgraan (68%). Geen betekenisvolle verskille is waargeneem in die rumen afbreekbare tempo tussen sojaboonoliekoekmeel en DDGS nie.

In die tweede studie (Hoofstuk 4) was daar ondersoek ingestel hoe die verskillende proteïenbehandelingsdiëte groei- slag en winsgewendheidsparameters van voerkraallammers

beïnvloed. Die behandelingsdiëte is geformuleer om iso-stikstof, iso-energie te wees en het een van vyf verskillende proteïenbronne bevat, sojaboonoliekoekmeel, DDGS, katoensaadoliekoekmeel, gedroogde brouersgraan en die laagste koste optimale formulering. Vyf-en-dertig Dohne Merino hammels was in ‘n heeltemal ewekansige ontwerp ingedeel met vyf behandelings en sewe herhalings. Geen betekenisvolle verskille is waargeneem in finale liggaamsmassa en voerdoeltreffendheid as gevolg van die dieetbehandeling nie. Karkas massas het ook nie verskil nie ($P > 0.05$). Ten slotte het hierdie navorsing bepaal dat DDGS inderdaad sojaboonoliekoekmeel ekonomies kan vervang as 'n proteïenbron om in voerkraalrantsoene te gebruik, en 'n waardevolle voerbron vir boere en voervervaardigers kan wees.

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- The Lord, for loving and sustaining me and for giving me the ability, strength, and endurance.

NOTE

The language and referencing style used in this thesis are in accordance with the requirements of South African Journal of Animal Science. This thesis presents a compilation of manuscripts where each chapter is an individual entity. It should be known that each chapter has its own reference list instead of one comprehensive list appearing at the end of the thesis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Ruminants, both wild and domesticated, have been a food source, throughout the history of humankind (Mann, 2018). However, in recent decades, there is a worldwide trend of higher poultry meat consumption, replacing ruminant meat products (Broderick, 2018; BFAP, 2018). Ruminants, in terms of meat production, are the least efficient of the major farm animals in converting feed into protein and energy for human use as shown in Figure 1.1 (Gillespie & Flanders, 2010).

It is a known fact that the feed conversion ratio (FCR) of poultry is significantly superior compared to cattle and sheep. In 2013, South African broiler producers achieved a FCR of 1.7 which was below the global sample average of 1.8 (Davids *et al.*, 2016). The FCR of lambs ranges between 4 and 6 depending on diet concentrate level and roughage quality (Brand & Franck, 1991; Fahmy *et al.*, 1992).

According to Wilkinson (2011), using such comparisons are gross over-simplifications and also do not take into account that ruminants can utilise land and feed resources that are not edible by the human population. A better comparison is to consider the proportion of feedstuffs utilised by different livestock species, which could have been potential human food. Using this system of measurement, ruminants are more efficient in the net production of human-food protein (Broderick, 2018). The reason for this is that poultry, as well as swine, consume feed ingredients humans could have consumed directly (Broderick, 2018).

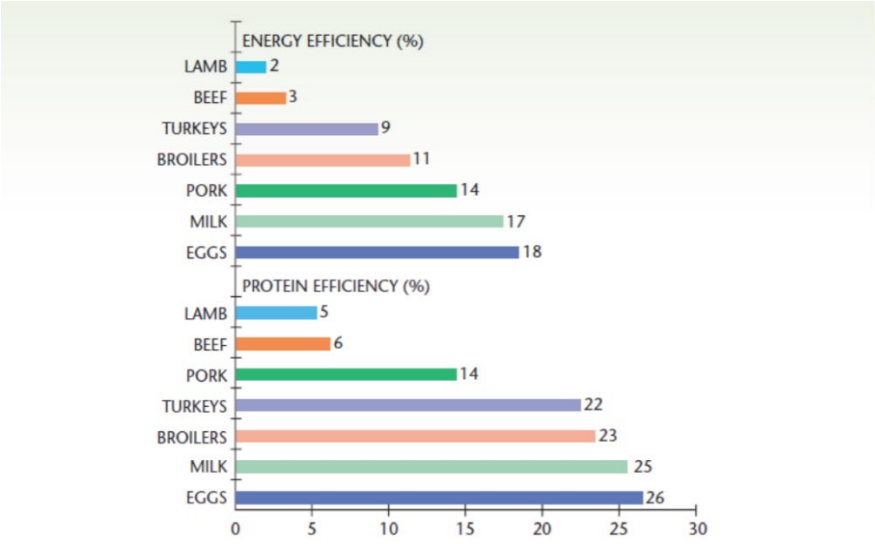


Figure 1.1 A comparison of the efficiency of major farm livestock in converting feed calorie intake to food calories output (energy efficiency) and converting crude protein in feed into edible protein in the form of meat, milk, and eggs (protein efficiency) (Gillespie & Flanders, 2010).

Considering intensively reared livestock, there is a continuous drive to increase feed efficiency. High nutrient-dense diets, derived from cereal grain crops and residues from the removal of oilseed crops (oilcake), especially soya bean meal are therefore fed (Wilkinson, 2011). Soya, however, can also be used directly by humans and the increased cultivation thereof often contributes to environmental issues, for example, deforestation in the Brazilian Amazonian region for soy cultivation (Garnett, 2009). Oilcake meals are an important protein component of feedlot diets, and soya meal is by far the most used protein ingredient to livestock diets globally (Chadd *et al.*, 2002). It is therefore important to seek alternative protein sources, with a low impact on the environment, economically feasible and, sustainable.

1.1. THE IMPORTANCE OF PROTEIN TO PRODUCTIVE RUMINANTS

Traditionally, protein is one of the most important components in ruminant nutrition (Parisi *et al.*, 2020). Often roughage and energy components are produced by livestock producers themselves. This implies that feed manufacturers have to constantly investigate innovative and sustainable protein ingredients to be competitive (Henchion *et al.*, 2017). Protein supplements are also a very costly component of the diet (Parisi *et al.*, 2020) and the importance of allocating high-quality protein sources that is economically viable, reliable, and sustainable, is the main focus (DiLorenzo & Galyean, 2010; May *et al.*, 2010; Hales *et al.*, 2016). These factors have motivated feed manufacturers and the livestock industry to search for sustainable and economical alternate feed

sources (Welker *et al.*, 2014). Wilkinson (2011) suggests that the sustainability of livestock sectors should be designed by the conversion of human-inedible inputs to human-edible animal proteins. An example of such an alternative is distiller's dried grains with solubles (DDGS) (Buenavista *et al.*, 2021). It is an outstanding and potentially lower cost feedstuff compared to other more conventional feed sources supplied to the livestock industry (Pecka-Kielb *et al.*, 2017). Distiller's dried grains with solubles is the dried residue that remains after the fermentation of grain (maize, wheat, sorghum, and barley) mash by selected yeasts and enzymes resulting in the end products ethanol and carbon dioxide (Lim *et al.*, 2011; Buenavista *et al.,* 2021).

The continuous growth in the number of processing plants that utilize maize to produce ethanol has increased the availability of by-product feeds. According to Popp *et al.* (2016) global ethanol and biodiesel production are both expected to increase to reach almost 135 and 39 billion litres respectively by 2024.

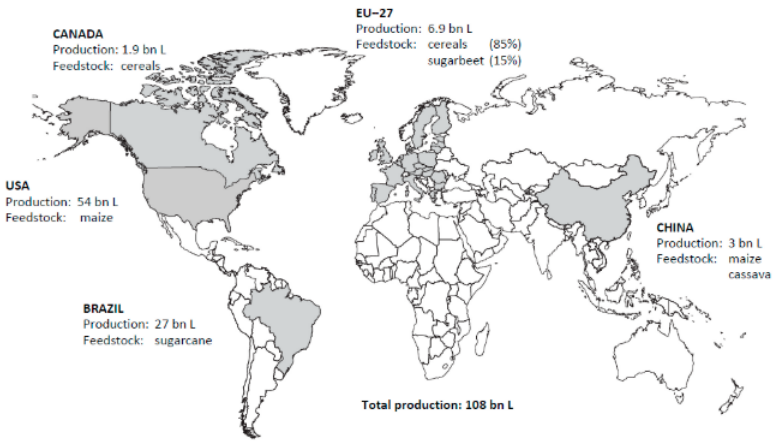


Figure 1.2 Word average fuel ethanol production; 2012–2014 (Popp *et al.*, 2016).

By-products produced from the ethanol industry have therefore become mainstream commodities, especially in the beef feed industry in the USA (Klopfenstein *et al.*, 2008). For various reasons, however, the sheep industry has been slower to include these feedstuffs (Schauer *et al.*, 2008; Borzuta *et al.*, 2014). A possible reason for that is that information on feeding ethanol by-products to sheep is less available in search results and educational publications (Pezzanite *et al.*, 2006) and the relative low sheep numbers in the USA.

1.2. RATIONALE OR PURPOSE OF THE STUDY

A study was done at the University of Stellenbosch to evaluate alternative protein sources to replace soybean meal (SBM) in sheep fattening diets. These sources include cotton meal, soybean meal, canola meal, dried brewers' grains and DDGS.

1.3. THE OBJECTIVES OF THE STUDY

1.3.1. **Trial 1:**

The study aimed to evaluate the extent and rate of ruminal protein degradation of the different protein sources against DDGS in an *in vitro* study.

1.3.2. **Trial 2:**

The objective of this secondary *in vivo* part of the study was to evaluate the effect of the different protein sources on growth parameters, carcass characteristics and economy of feedlot lambs.

1.4. HYPOTHESIS

The production response of feedlot lambs will be similar when fed rations formulated with different protein sources, provided their protein, energy, vitamin, and mineral requirements are being met.

1.5. RESEARCH CONTRIBUTION

The outcomes of this study would inform feed manufacturers about the effect of ruminant diet formulation using co-product feed ingredients as alternative protein sources. This data can be used to improve animal performance efficiencies and to lower the unit cost of production which is critical for economic sustainability in ruminant animal production.

1.6. REFERENCES

Borzuta, K., Borys, B., & Lisiak, D. 2014. Quality of meat, fatty acid profile, and slaughter value of lambs intensively fed feed supplemented with biofuel co-products. Food. Sc Technol Qual 6, 112–124.

Brand, T. S., & Franck, F. 1991. Wheat-straw as roughage component in finishing diets of growing lambs. S. Afr. J. Anim. Sci. 21, 184-188.

Broderick, G. A. 2018. Review: Optimizing ruminant conversion of feed protein to human food protein. Animal 12, 1722–1734.

Buenavista, R. M. E., Siliveru, K., & Zheng, Y. 2021. Utilization of Distiller's dried grains with solubles: A review. J. Agric. Food Res. 5 https://doi.org/10.1016/j.jafr.2021.100195.

Bureau for Food and Agricultural Policy (BFAP). 2018. Baseline Agricultural outlook 2018-2027, University of Pretoria.

Chadd, S. A., Davies, P., & Koivisto, J. M. 2002. Practical production of protein for food animals. In Protein sources for the animal feed industry. Cirencester, Gloucestershire.

Davids, T., Delport, M., Gouse, M., Gxotiwe, V., Kalaba, M., Louw, M., Meyer, F., Scheltema, N., Joubert, C., Ngqangweni, S., Nyhodo, B., Parfitt, D., Van Der Walt, S., & Verwey, R. 2016. Evaluating the competitiveness of the South African broiler value chain.

DiLorenzo, N., & Galyean, M. L. 2010. Applying technology with newer feed ingredients in feedlot diets: Do the old paradigms apply? J. Anim. Sci. 88, E123–E132.

Fahmy, M. H., Boucher, J. M., Poste, L. M., Gregoire, R., Butler, G., & Comeau, J. E. 1992. Feed efficiency, carcass characteristics, and sensory quality of lambs, with or without prolific ancestry, fed diets with different protein supplements. J. Anim. Sci. 70, 1365–1374.

Garnett, T. 2009. Livestock-related greenhouse gas emissions: impacts and options for policy makers. Environ. Sci. Policy 12, 491–503 https://doi.org/10.1016/j.envsci.2009.01.006.

Gillespie, J. R., & Flanders, F. B. 2010. Modern Livestock and Poultry production 8th ed. Delmar.

Hales, K. E., Shackelford, S. D., Wells, J. E., King, D. A., Pyatt, N. A., & Freetly, H. C; & Wheeler, T. L. 2016. Effects of dietary protein concentration and ractopamine hydrochloride on performance and carcass characteristics of finishing beef steers. J. Anim. Sci. 94, 2097–2102.

Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., & Tiwari, B. 2017. Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. Foods 6, 1–21 https://doi.org/10.3390/foods6070053.

Klopfenstein, T. J., Erickson, G. E., & Bremer, V. R. 2008. Board-invited review: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86, 1223–1231 https://doi.org/10.2527/jas.2007-0550.

Lim, C., Li, E., & Klesius, P. H. 2011. Distiller's dried grains with solubles as an alternative protein source in diets of tilapia. Rev. Aquac. 3, 172–178.

Mann, N. J. 2018. A brief history of meat in the human diet and current health implications. Meat Sci. 144, 169–179 https://doi.org/10.1016/j.meatsci.2018.06.008.

May, M. L., DeClerck, J. C., Leibovich, J., Quinn, M. J., DiLorenzo, N., Smith, D. R., Hales, K. E., & Galyean, M. L. 2010. Corn or sorghum wet distillers grains with solubles in combination with steam-flaked corn: *in vitro* fermentation and hydrogen sulfide production. J. Anim. Sci. 88, 2425–2425 https://doi.org/doi: 10.2527/jas.2009-2486. Epub 2010 Apr 9. PMID: 20382867.

Parisi, G., Tulli, F., Fortina, R., Marino, R., Bani, P., Dalle Zotte, A., De Angeli, A., Piccolo, G., Pinotti, L., Schiavone, A., Terova, G., Prandini, A., Gasco, L., Roncarati, A., & Danieli, P. P. 2020. Protein hunger of the feed sector: the alternatives offered by the plant world. Ital. J. Anim. Sci. 19, 1205–1227 https://doi.org/10.1080/1828051X.2020.1827993.

Pecka-Kielb, E., Zachwieja, A., Mista, D., Zawadzki, W., & Zielak-Steciwko, A. 2017. Use of Corn Dried Distillers Grains (DDGS) in Feeding of Ruminants.

Pezzanite, L., Student, A. S., Gunn, P., Neary, M., Small, E., Specialist, R., Lemenager, R., Specialist, E. B., Lake, S., & Specialist, E. B. 2006. Value of Distiller's Grains as a Feed for Sheep.

Popp, J., Harangi-Rákos, M., Gabnai, Z., Balogh, P., Antal, G., & Bai, A. 2016. Biofuels and their co-products as livestock feed: Global economic and environmental implications. Molecules 21, 1–26.

Schauer, C. S., Stamm, M. M., Maddock, T. D., & Berg, P. B. 2008. Feeding of DDGS in lamb rations. Sheep Goat Res. J. 23, 15–19.

Welker, T. L., Lim, C., Barrows, F. T., & Liu, K. 2014. Use of distiller's dried grains with solubles (DDGS) in rainbow trout feeds. Anim. Feed Sci. Technol. 195, 47–57.

Wilkinson, J. M. 2011. Re-defining efficiency of feed use by livestock. Animal 5, 1014–1022.

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION

Worldwide the demand for meat is increasing as the global population is growing (Henchion *et al.*, 2014). The red meat industry is also one of the South African agricultural sectors that shows similar increases. During 2017/18, this industry contributed 18,4% towards the gross product value of agricultural production in South Africa (DAFF, 2019). During 2018 in South Africa, meat represented 35% of the expenditure on the consumer food expenditure component compared to 24% on bread and grains, 13% on fruit and vegetables (including potatoes), and 13% on milk, milk products, and eggs, respectively (DAFF, 2019).

Roughly 80% of South African agricultural land is suitable for extensive livestock farming but the recent droughts have negatively affected the grazing area of approximately 590 000 km^2, representing 53% of all agricultural land (DAFF, 2020). The sheep farms within South Africa are clustered in the more arid regions of the country, i.e., Northern Cape, Eastern Cape, Western Cape, Free State, and Mpumalanga provinces (Figure 2.1). According to DAFF (2018a), there are approximately 8 000 commercial sheep farms throughout the country and about 5 800 communal farmers. Almost 80% of communal sheep are found in the Eastern Cape (Cloete & Olivier, 2010). The total number of sheep in South Africa at the end of August 2019 was projected to be 22,06 million (DAFF, 2020). These figures represent a 1,96% reduction compared to the estimated 2018 figures of 22,50 million (DAFF, 2020).

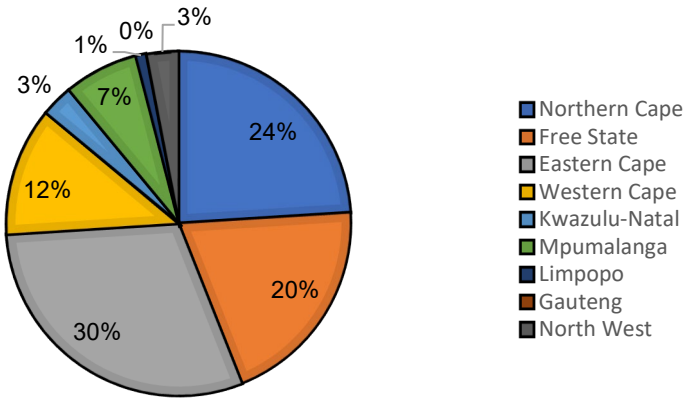


Figure 2.1 Sheep distribution in all provinces of South Africa (DAFF, 2020).

Currently, South Africa is a net importer of mutton and lamb, with the main exporters to South Africa being Australia, New Zealand, and Namibia (Figure 2.2). In some countries (mostly developed countries) from which livestock products are imported, subsidies are paid to their producers and often even the export refunds as well, thus creating a distortion in the market (Meissner *et al.*, 2013). Despite this, current trends are showing a decline in South African imports which could be attributed to increased international prices (DAFF, 2018a).

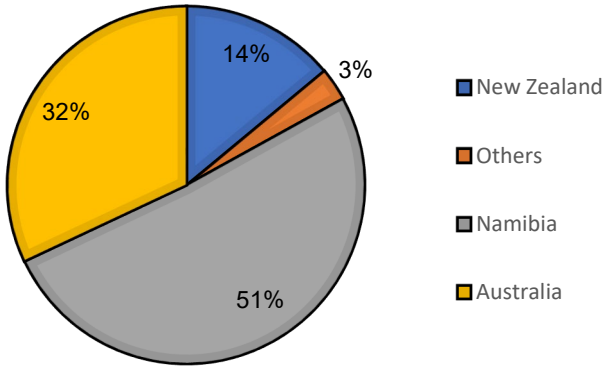


Figure 2.2 Countries of origin for mutton and lamb imports to South Africa (DAFF, 2018a).

Local production has increased and fluctuated between 110 and 160 thousand tonnes between 2002 and 2010 (Figure 2.3). A sharp decline in sheep production during 2010 (Figure 2.3) coincided with the outbreak of Rift Valley fever. Pienaar and Thompson (2013) reported that a total of 484 outbreaks were confirmed during the 2010 season with 14342 animal cases and 8877 animal deaths. Consequently, significant flock reductions were seen through 2015 because of a Foot-and-mouth disease outbreak (Hoskin, 2015). The South African livestock industry also had to recover from the impact of a devastating drought with an estimated reduction in the national livestock herd in South Africa of 15% during 2015 (Mare *et al.*, 2018). Flock rebuilding takes time and coupled with prolonged drought conditions in the Western Cape, which contribute approximately 12% of national ovine production, the process is further constrained (BFAP, 2018). In 2019, foot-and-mouth disease broke out in Limpopo resulting in the World Organisation for Animal Health temporarily suspending SA's disease-free zone status. This had led to a ban on exports of animal products from South Africa (Phakathi, 2020).

In South Africa, sheep farmers are also particularly vulnerable to stock theft compared to cattle farmers. Additionally, predators, including the black-backed jackal (*Canis mesomelas*) and caracal (*Felis caracal*) are also perceived to have a major impact in extensive regions (Cloete & Olivier, 2010). Livestock farmers diversifying into alternative extensive rangeland livestock

production systems such as game farming further led to a decline in sheep numbers (Van Wyk, 2011). The head of ABSA Agribusiness, Janvosky (2016), claimed that there are 6330 exempted privately-owned wildlife farms which equate to approximately 14,7 million hectares. In the Eastern Cape alone, where extensive rearing of livestock is the main form of land use, substantial numbers of conversions has occurred from commercial livestock production to game farming (Chiyangwa, 2018). According to Pasmans & Hebinck (2017), 12% of the Eastern Cape has been converted to game farming since 1996.

The indication that the sheep industry is sustainable is confirmed by the inclination to import mutton and lamb (Figure 2.3). The per capita consumption of lamb and mutton, however, is declining and is currently just above 3 kg/capita/year (Figure 2.4). Consumer demand for livestock products is based on the price, availability, disposable income and the price of alternatives (pork or poultry). When prices increase, the consumer often migrates to different dietary protein sources. Being healthy (the substantial amount of unsaturated fats is mainly found in the skin and can easily be removed (Marangoni *et al.*, 2015) and the most affordable option, the consumption of chicken is currently still the highest. Poultry meat is projected to expand by 27% over the next 10 years (BFAP, 2018). The consumption of pork and beef are also projected to expand by 24% and 23%, respectively, over the same period (BFAP, 2018). Mutton, the most expensive meat option, is only projected to expand by 11% by 2027 (BFAP, 2018).

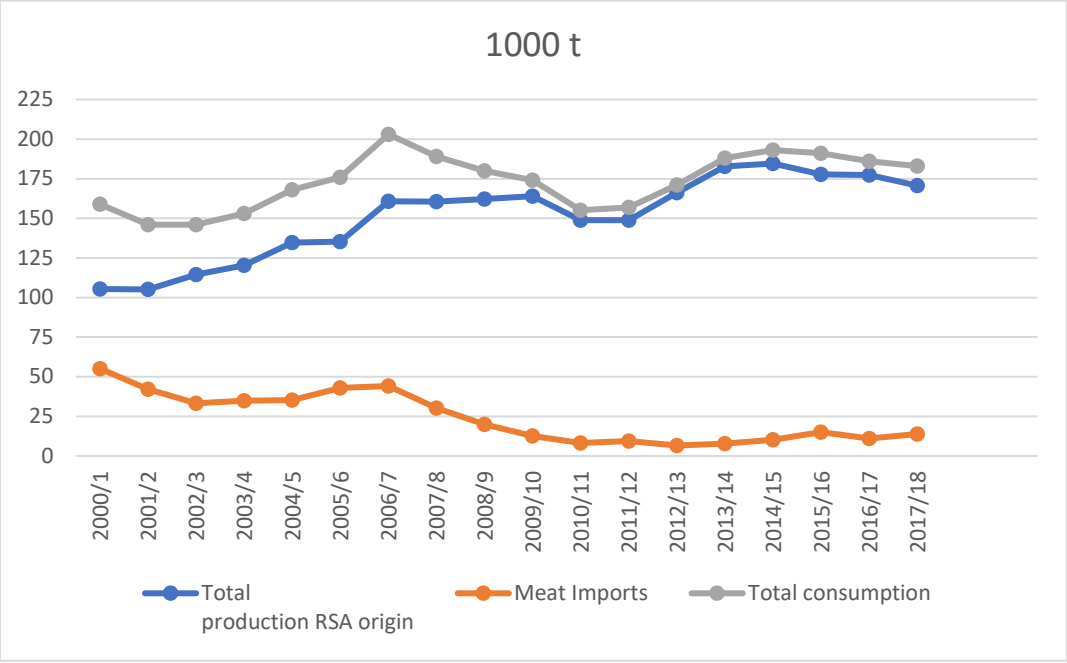
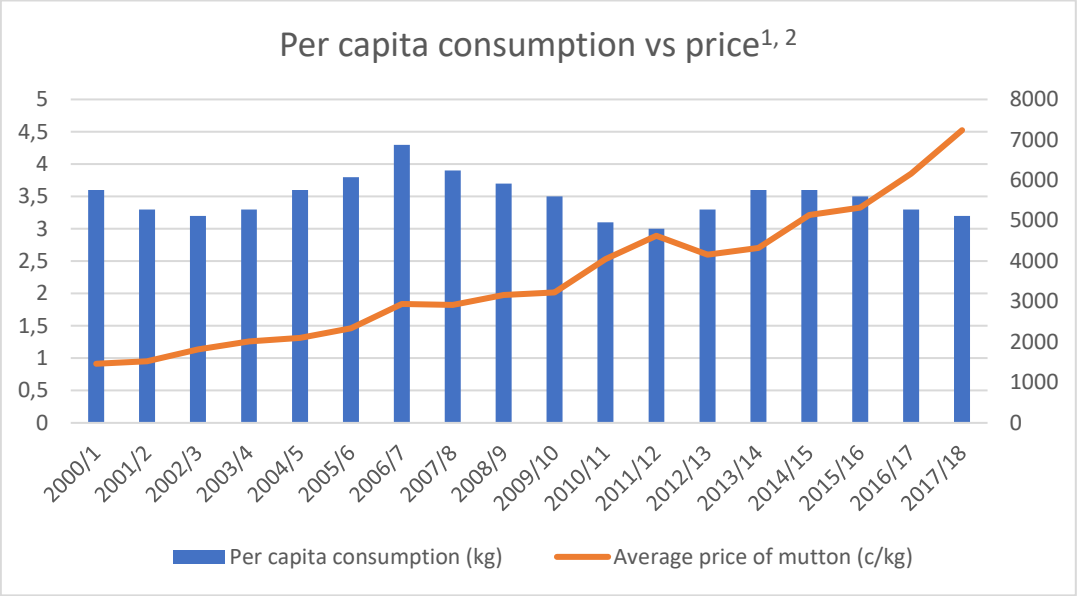


Figure 2.3 Sheep, lamb and goat: production numbers, imports, and total consumption in South Africa (Abstract of Agricultural Statistics, 2019).



1. Purchase price of chilled carcasses, including the fifth quarter
2. Up to 2003/04, average auction price of mutton on the hook at certain auction markets

Figure 2.4 Per capita consumption of sheep, lamb and goat in South Africa versus carcass price (c/kg) ('Abstract of Agricultural Statistics', 2019).

2.2. THE SOUTH AFRICAN SHEEP INDUSTRY

2.2.1. **Sheep production systems in South Africa**

The sheep industry in South Africa ranges from the extensive arid rangeland where the carrying capacity is less than one large stock unit per 40 ha to fairly intensive pasture-cropping regions (Meissner *et al.*, 1983). During droughts, especially in the case of extensive rangeland conditions, the number of lambs produced depends on feed availability and nutrient quality (Abraham *et al.*, 2019). In extensive production systems, the lambing season is primarily determined by the natural growing season to provide the ewes with sufficient natural veld to raise their lambs (Terblanche, 2013). Raising lambs on grass or natural veld normally results in slower growth rates, generally because no hormonal growth implants and concentrate feeds are used, and feed supplements are limited to strategic mineral, energy, and protein supplements (Webb & Erasmus, 2013). In the winter rainfall region of South Africa where cereal is produced, many farms produce autumn lambs (Brand & Brundyn, 2015). Pregnant and lactating ewes are dependent on the low-energy and low-protein crop residues in summer and early autumn until the first rains fall (Brand & Brundyn, 2015). However, these stubble rests have a very low nutrient value (Brundyn *et al.*, 2008).

In the study by Brand & Brundyn, (2015) they found by providing the nursing lambs with creep feed and supplementing their dams resulted in lambs that reached slaughter condition at an earlier age and the ewes had a decrease in live weight loss.

According to Arnold *et al.* (1979) in the absence of human interference, lambs will naturally wean between 100 and 180 days of age. However, in modern production settings, natural weaning rarely occurs and is performed due to several factors, for example, production systems or pasture and feed availability (Campbell *et al*., 2017), the breed of the sheep and if they are marketed directly of the veld or placed in a feedlot for finishing (Terblanche, 2013). It is common practice with intensive systems, to wean lambs earlier (90 to 100 days) and with an accelerated lambing production system (e.g., 8-month system) or when nutritional conditions are poor, lambs can be weaned as early as 60 to 90 days (Cloete *et al.*, 2000). While in extensive systems lambs are weaned later (up to 150 days) (Terblanche, 2013).

In South Africa, the majority of lambs and older sheep are fattened and marketed directly from the pasture or veld, while a small percentage of lambs are fattened for short periods in feedlots to warrant consistent carcass weights and quality (Webb & Erasmus, 2013). With lower levels of lamb production (DAFF, 2020) from the traditional extensive systems, because of limited pasture availability, an inclination to shift to more intensive feeding systems might occur. This is to exploit increased producer prices.

In the case of intensive systems more cultivated pastures are used to increase the carrying capacity per hectare, or by placing animals in feedlots to increase animal numbers (Landman, 2013). A higher degree of flock and nutritional management is associated with intensive systems compared to extensive systems (Simões *et al.*, 2021). Innovative feeding strategies are used and changes to the traditional breeding systems, for example allowing practices of accelerating lambing, are made to improve lambing percentages and weight gains. Accelerated lambing refers to ewes lambing more frequently than once per year (Dally & Hohenboken, 1979). This type of intensive reproduction management can bring down the maintenance cost of the breeding stock per offspring reared, as well as provide a constant supply of lambs throughout the year (Landman, 2013). The accelerated lambing systems commonly used are three lamb crops per ewe every two years and five lamb crops per ewe every three years in the STAR breeding system (DeNicolo, 2007).

Much of the Southern African sheep and grain regions complement each other with the sheep utilizing crop residues and also legume rotation crops (Cloete & Olivier, 2010). The small grain production regions like the Overberg and Swartland that have ready access to lambs and feed as well as being climatically suitable are well-positioned to capitalise on feedlotting. Feedlotting allows producers to maintain production and to achieve rapid growth when feed prices are low, as well as generate cash flow (Duddy *et al.*, 2016).

2.2.2. **Feedlotting lambs**

The lamb feedlot industry is a relatively new industry and guidelines for intensive feeding are not as well defined for sheep when compared to intensive finishing systems for the beef, pork, and poultry industries (Van der Merwe, 2020). Therefore this leads to a largely unexplored value-added opportunity for the sheep industry (Male, 2012). The purpose of feedlot finishing is to take advantage of the superior growth rates and feeding efficiencies of young animals by rearing them to desirable market weight for optimal profitability (Van der Merwe *et al.*, 2020).

There are mainly three types of feeding systems used in sheep feedlots:

- Pelleted diets, commonly used with self-feeders (Li *et al.*, 2021);
- Total mixed rations (TMR) using open troughs;
- Feeding whole grain and roughage separately in cafeteria systems (Rodríguez *et al.*, 2007).

These types of systems depend on infrastructure and labour that is available. The cheapest and simplest system is to feed grain and hay separately, which allows the sheep to select their diet, but it has the potential risk of the lambs consuming more roughage than in the case with the pellets or TMR feeding systems, resulting in lower growth rates and feed conversion efficiency (Bowen *et al.,* 2006). Furthermore, a too high intake of concentrate to roughage might also induce rumen acidosis (Hernández *et al.*, 2014; Duddy *et al.*, 2016).

Atwood *et al.* (2001) suggested that cattle can regulate their intake and consume a correct ratio of protein to energy, relatable to a scientifically balanced ration when accustomed to grain. The latter experiment consisted of calves that had free-choice roughage and grain (barley, maize, and oats) each ingredient fed separately in four adjacent bunks. The noticeable variation in intake of feed, from day to day, and among individuals proposed that by providing a choice, animals will discriminate and change preferences constantly (Atwood *et al.,* 2001). The author allowed the calves to have continuous access to creep feed in addition to either a dry-lot forage mix or pasture before the study commenced. Commonly, livestock producers believe that animals will overeat grain if they have the option of free choice grain leading to acidosis. This belief is the reason why total mixed rations are often preferred. Work done by Forbes & Kyriazakis (1995) with sheep indicated similar behaviour. When offered both concentrate and long forage, sheep will consume significant amounts of forage, even though it requires more time and energy to chew and digest as well as being low in nutrient density (Forbes & Kyriazakis, 1995). Using pelleted feed results in less selective feeding behaviour and wastage while the use of self-feeders is also possible. Pelleted feed normally come at a significant cost disadvantage (Li *et al.,* 2021). With TMR systems, grinding and mixing equipment is required as well as regular feeding is required (Bowen *et al.*, 2006).

One of the main drivers to move lambs to feedlots is to remove all non-reproductive animals from the pasture/veld allowing higher reproductive ewe numbers on a farm (Van Niekerk, 2020). This is even more pronounced when quality pasture is unavailable or during drought conditions (Van Niekerk, 2020). By weaning the lambs earlier, ewes can gain condition faster especially in

accelerated lambing systems, where the time available before the ewe needs to be joined with the rams are limited (Lewis *et al.*, 1996). If grain production forms part of the operation, feedlotting allows value-adding to grain income (Rusche, 2020). When lamb prices are high relative to high feed prices, it may be a more viable option to sell the lambs to commercial feedlots (Male, 2012).

Risks associated with grain finishing of lambs include mortalities, shy feeders, and unexpected changes in market prices for lambs and feed (Duddy *et al.*, 2016). It is a low-margin business and therefore exceptional business management skills and good husbandry is essential (Larson, 2017). Animal associated stress factors include adaption from mainly forage-based diets to intensive confined environments with high concentrate diets. Transport also add to heightened stress levels (Rice *et al.*, 2016). This is especially significant in the first two weeks during the adaption period, which is the most important time to ensure optimal feedlot performance (Stanton & LeValley, 2006). Too rapid introduction of grain to the diet or change from roughage to high starch grains will lead to the accumulation of lactic acid causing acidosis (MLA, 2007). This adapting phase range from anything between five to 15 days before the lambs are onto the finishing diet (Stanton & LeValley, 2021). This gradual introduction to concentrate feed is important as the major cause of mortalities in feedlots is acidosis when lambs are transitioned too fast to high starch feed (Male, 2012). It is also important to keep suitable crude fibre levels and include buffers in the feed (Bello *et al.*, 2016). The aim is to get lambs onto a full finishing diet as quickly as possible while avoiding acidosis, as this will have a significant impact on profitability (Larsen, 2017). Mcdonald *et al.* (1988) confirmed the advantage of exposing lambs to feedstuffs before introducing them to intensive feeding systems, resulting in increased feed intake during the first five days of trough feeding and fewer animals that are reluctant to eat concentrated rations during this period. They suggested that it is advantageous to expose young sheep to dietary grain early in life, e.g., creep feed, and should be part of best-practice management in preparing animals for feedlot adaption. According to Bello *et al.* (2016) apart from rumen health and suitable animal welfare conditions, other priority production approaches for feedlots includes the following:

2.2.2.1. Feed Efficiency

Feed conversion ratio (FCR) is defined as the feed requirement in kg per kg body weight gain (Wenk *et al.*, 1980; Lima *et al.,* 2017). This principle involves achieving high body weight gain with the lowest feed intake possible, reaching a suitable carcass fat content and meat quality (Bello *et al.*, 2016). Definitions of the indicator trait of feed efficiency can be seen in Table 2.1. The feed cost can represent approximately 70% of the total amount in confinement and to mitigate this cost, besides using cheaper food, is using more efficient animals (Lima *et al.*, 2017). A feedlot study done by Van der Merwe *et al*. (2020), comprised of the following experimental groups; the Dohne Merino (ewe =10, ram =6), Dormer (ewe =5, ram =5), Dorper (ewe =6, ram =8), Meatmaster (ewe =12, ram =12), Merino (ewe =6, ram =6), Namaqua Afrikaner (ewe =8, ram =6) and South African Mutton

Merino (SAMM) (ewe =4, ram =6). They were fed the same diet (10.41 MJ ME/kg feed and 19.06% crude protein on an as fed basis) and their feed intake and weekly growth were monitored, from an initial weight of 30 kg. The results indicated high growth rates from Dormer, SAMM, Dohne Merino, Meatmaster, and Dorper lambs; with the Dormer lambs demonstrating exceptionally high growth rates (Van der Merwe *et al*., 2020). In the latter study, the authors concluded that the feeding efficiency of most of the breeds (apart from Merino and Namaqua Afrikaner lambs; average daily gain (ADG) <300 g/day) in a feedlot proved to be favourable. In order to manage the risks and remain profitable, lambs confined within a feedlot system are generally required to grow above 300 grams per day (Jolly & Wallace, 2007).

Table 2.1 Definition of the indicator traits of feed efficiency (Adapted from Lima *et al.,* 2017).

Trait	Formula[1]	Definition
Feed conversion ratio (FCR; kg DM/kg) Relative	$\frac{DMI}{ADG}$	Amount of feed consumed divided by the weight gain. Lower values are favourable.
Relative growth rate (RGR; kg BW/day	$100 \times \left[\frac{(log\ BWf - logBWi)}{Days\ in\ test}\right]$	Growth potential in relation to degree of maturity. Higher values are favourable.
Kleiber's ratio (KR; g gain/kg $BW^{0.75}$)	$100 \times \left[\frac{ADG}{BW^{0.75}}\right]$	ADG, in grams, proportional to each kilogram of metabolic weight. Higher values are favourable.
Residual feed intake (RFI; kg DM/day)	$DMI\ -\ DMIe\ ADG$	Difference between observed and estimated DMI based on ADG and $BW^{0.75}$. Lower values are favourable
Residual weight gain (RWG; kg gain/day)	$ADG\ -\ ADGe$	Difference between observed and estimated ADG based on DMI and $BW^{0.75}$. Higher values are favourable.
Residual intake and BW gain (RIG)	$RWG\ +\ [(-1)\ \times\ RFI]$	Simple index including RFI and RWG whose variance is adjusted at 1. Higher values are favourable.

[1] DM - Dry matter; ADG - average daily weight gain; DMI - dry matter intake; BW - body weight; BWf - final BW; BWi - initial BW; BW0.75- metabolic BW; DMIe – estimated DMI; ADGe - estimated ADG

2.2.2.2. Meat and carcass quality

Keeping the genotype of the sheep in mind, the feed strategy should focus on achieving a suitable fat content in the carcasses. Professional carcass classification is performed at registered abattoirs to ensure optimum fat cover and distribution (Webb, 2015). In South Africa, a carcass classification system is used, which is based on physical and compositional attributes, this include age (age categories A, AB, B, C) (based on dentition), carcass fatness (carcass fat codes 1-6) and carcass conformation (carcass conformation codes 1-5), and damage (1 – 3) (Government Notice No. R863, 2006) (Table 2.2).

Table 2.2 South African carcass classification system for cattle and small stock. (Government Notice No. R863, 2006).

Descriptor	Classification	Guideline	Identifier
Animal age	A	No permanent incisors	Roller mark with purple ink
	AB	At least one, but not more than two permanent incisors	Roller mark with green ink
	B	At least three, but not more than six permanent incisors	Roller mark with brown ink
	C	More than six permanent incisors permanent incisors	Roller mark with red ink.
Carcass fatness*	0	No fat, 0 mm (< 1.0% subcutaneous fat)	Fat class indicated along with age roller mark.
	1	Very lean, < 1 mm (1.0–5.6% subcutaneous fat)	
	2	Lean, 1–4 mm (5.6–8.6% subcutaneous fat)	
	3	Medium, 4–7mm (8.6–11.6% subcutaneous fat)	
	4	Fat, 7–9mm (11.6–14.6% subcutaneous fat)	
	5	Slightly overfat, 9–11 mm (14.6–17.6% subcutaneous fat)	
	6	Excessively overfat, > 11 mm, (> 17.6% subcutaneous fat)	
Carcass conformation	1	Very flat	Stamp with conformation score on side of carcass.
	2	Flat	
	3	Medium	
	4	Round	
	5	Very round	

*Subcutaneous fat measured between the third and fourth lumbar vertebrae, 25 mm from the midline of the carcass

The age category is important because it give an indication of tenderness, with meat from older animals having lower collagen solubility, resulting in tougher meat (Bruwer et al., 1987). Fatness classes are also important, indicating quality factors relating to juiciness and flavour (Bruwer et al., 1987). *In vivo* ultrasound measurement of the animal can be used to predict fat depth on the *longissimus lumborum* muscle (Berg *et al.*, 1996). A lean-to-medium-fat carcass is described as having subcutaneous fat depth ranging between 1 mm to 7 mm (Department of Agriculture, 2006). Owens *et al*. (1993) considered mature size (weight) as the point of maximum protein weight despite the increased fat deposition that can occur beyond this point. This is important to know because the onset of fat deposition does influence the rearing times and marketing weight of lambs from the different breeds (Berg *et al.*, 1996). Between breeds, the time in reaching mature weight differs, and this needs to be taken into consideration to decide the slaughter time (Van der Merwe, 2020).

In the feedlot industry where many animals often are slaughtered at a fixed number of days in the feedlot rather than when they reach a fat-constant basis, later maturing genotypes will be leaner (Toldra, 2017). The ratio of lean to fat tissue gain in an animal over a period can be used as a measure of maturity (Casey & Webb, 2010). This bodyweight critical point is when body weight increases normally shift towards fat tissue gain instead of lean tissue gain. Considering the maturity of South African sheep breeds, SA Mutton Merino, SA Merino, Dorper and Pedi (fat-tail breed) reaches this critical point at bodyweights of 42.3, 27.8, 27.2 and 22.2 kg, respectively, suggesting that these breeds could be ranked from late to early maturing genotypes (Casey & Webb, 2010). The application of different feeding regimes for finishing slaughter lambs, in particular for early maturing breeds, may be necessary (Van der Merwe, 2020; Van der Merwe *et al.*, 2020). Reducing the energy density in feed by using cheaper raw materials and feeding for a longer period are good strategies to achieve this aim (Sheridan *et al.*, 2003).

2.2.2.3. Carcass yield (%) improvement

Feedlot and abattoir operations usually try to improve profit by reducing fixed costs and achieving a higher amount of meat per slaughtered animal (Louw *et al.*, 2018). Slaughtering on a fat-constant basis, therefore, implies that later-maturing genotypes will produce heavier carcasses (Toldra, 2017). Van der Merwe (2020) demonstrated that different breeds grow at different rates ($P < 0.05$). Each breed differed in age at the same body weight of 30 kg where Namaqua Afrikaner lambs were significantly the oldest (145 days), followed by the Meatmaster (101 days), Merino (91 days), and Dorper (89 days) lambs, compared to the younger Dormer and SAMM lambs (74 and 71 days), respectively (Van der Merwe, 2020). In South Africa, price premiums are paid for the most sought after classification lamb carcasses of A2 or A3 grade, weighing between 18 and 22 kg Cloete et al., 2000). Early maturing breeds, especially the fat-tailed breeds and Dorper (under intensive or favourable environmental conditions), need to be in the feedlot for shorter periods and attain lighter market weights (live weight of 32-39 kg) to prevent being classified over fat, which unfortunately

leads to lower carcass weights per slaughtered animals (Van der Merwe *et al.*, 2020). Cloete et al. (2000) also confirmed that it should be aimed to slaughter Dorper at lower live weights, possibly closer to 32-35 kg to avoid being penalised for being too fat. This early maturity characteristic of the Dorper is often discriminated against in commercial feedlot operations (Brand *et al.*, 2018). Later maturing Dohne Merino, Dormer, and SAMM lambs can be slaughtered at heavier final live weights (~44.4 kg) within 30-40 days of feeding resulting in it being more popular breeds with commercial feedlot enterprises (Cloete *et al.*, 2000).

2.3. DIGESTIVE PHYSIOLOGY AND PROTEIN DIGESTION IN SHEEP

Despite sheep and cattle both being ruminants, they do have different feeding requirements (Colucci *et al.*, 1989). Differences between them include physiological functions e.g. wool growth and also related to their body size which is 10 to 12 times smaller than cattle (Cannas *et al.*, 2019). Bodyweight (BW) and size have a strong correlation with feed intake, a cow does have a greater feed intake relative to a sheep (Hackmann & Spain, 2010). Allometric equations (Calder, 1984; Peters, 1983) can be used to quantify this relationship using the following equation (Hackmann & Spain, 2010):

$$\mathbf{Y=a \cdot BW^{b}}$$

Where: Y = value of a physiological parameter
a = allometric intercept (value of y at BW = 1)
b = scaling parameter.

Allometry refers to exponential scaling between a body part and the size of the body as a whole (Anzai *et al.*, 2017). Considering ruminants, the wet fermentation contents of the reticulorumen increase proportionally to body weight (BW) over time. Despite that, there is a lower proportional increase in energy requirements due to allometric scaling as a function of metabolic BW ($BW^{0.75}$) (Cannas *et al.*, 2019). According to the oldest and best-known power relationship, the Kleiber's law, the energy requirement increases proportionally less compared to body size due to their allometric scaling as a function of metabolic BW ($BW^{0.75}$) (Cannas *et al.*, 2019). This means that smaller ruminants have a higher maintenance requirement per kilogram of BW and a lower ratio between the reticulorumen volume and energy requirement than cattle (Cannas *et al.*, 2019).

Ruminants regurgitate their feed and have a special four-compartment stomach (rumen, reticulum, omasum, and the abomasum) that ferment ingesta before it reaches the lower intestines (NRC, 2007). Even though they have these four different compartments there are definite differences between ruminant species in how the gastrointestinal tract (GIT) functions (Rowe *et al.*, 1999). Goats

in contrast to sheep are predominantly browsers and will select shrubs but also can eat fibrous plants whereas sheep have very effective fermentation systems enabling them to graze high fibre pastures and ranges (Hofmann, 1989; Raoult *et al.*, 2021).

The reticulorumen is the largest compartment of the ruminant digestive tract, containing a complex anaerobic microbial community including, bacteria, archaea, protozoa, fungi, and viruses. Out of all of these organisms, bacteria make up the largest amount with an estimated 95% of the total microbial population (Pérez-Barbería, 2020). Bacteria also contribute the most to the feed, in regards to digestion and the conversion to volatile fatty acids and microbial protein (Loor *et al.*, 2016). The microbial species are influenced by the physical characteristics, composition, or quantity of the diet. The amino acid composition of the rumen undegradable protein (RUP) fraction of the diet is important because it complements the microbial protein synthesised to meet the requirement of the animal (Mercer *et al.*, 1980).

The rumen degradable protein (RDP) fraction of dietary crude protein is hydrolysed in the rumen to peptides and then to amino acids and ammonia by microorganisms, while ruminal undegradable protein (RUP) or bypass protein passes through the rumen undigested (Schwab *et al.*, 2003). Both types of protein are important to the ruminant (Schwab *et al.*, 2003). Rumen degradable protein is utilised by the microorganisms, to produce microbial crude protein (MCP) (Povey *et al.*, 2016). This MCP is then digested enzymatically in the lower digestive tract when passed to the lower intestines. The digested MCP portion together with the RUP portion is then absorbed in the small intestine to provide the animal with the required amino acids (Povey *et al.*, 2016). The level of this metabolizable protein (MP) that is available for absorption in the small intestine depends on the flow and digestibility of the MCP and RUP (NRC, 2001). During the degrading process of dietary protein and synthesising of MCP in the rumen, losses of 20% or higher occur (Agle *et al.,* 2010; Ruzic-Muslic *et al.*, 2014). This will lead to lower amounts of amino acids reaching the small intestine and therefore a reduced level available to the animal. These losses can create a situation where animal production can be impaired (Ruzic-Muslic *et al.*, 2014).

In the past, the crude protein (CP) system has been used to define ruminant protein requirements and feedstuff protein value (NRC, 1984). Crude protein is calculated by multiplying the amount of nitrogen in a feedstuff by 6.25. However, using only CP to formulate rations erroneously assumes that all protein is equally degraded in the rumen (NRC, 1996).

Broderick *et al.* (1988) specified that dietary proteins vary greatly in ruminal degradation. Proteins with low ruminal degradation are very valuable to ruminants and good responses have been reported with animals that have a high protein requirement (Khalid *et al.*, 2012). It is therefore important to quantify ruminal protein degradation of specific raw materials used in ruminant nutrition. As natural protein (normally high in RUP) sources are expensive, efficient utilization is of importance as it is not as efficiently used by ruminants when compared to monogastric animals (Brand & van der Merwe, 1993). According to Broderick (2018), several trials have been done by dairy scientists,

to assess the requirements for dietary crude protein (CP) and reported that dairy farmers often overfed CP. Earlier, Broderick (2003) concluded by incrementally increasing dietary crude protein from 15,1% to 16,7% and 18,4% (by adding solvent-extracted soybean meal to the diet at the expense of grain). An increase in milk and protein yield could only be shown with the 16.7% treatment. No additional production responses seemed to be present when CP from 16.7% to 18.4% increased (Broderick, 2003). When the amount of RDP exceeds the capacity of the rumen microbes to synthesize microbial protein, ammonia is produced (Jayawardena, 2000; Erickson & Kalscheur, 2020). The ammonia is absorbed through the rumen wall and transported via the portal system to the liver where it is converted to urea and excreted in the urine (Doranalli, 2010). See Figure 2.5. The conversion from ammonia to urea is done at the expense of an energy cost to the animal. With both the loss of dietary crude protein and energy it reduces the utilization efficiency of rumen degradable protein hence, reducing ruminant production (NRC, 2001). As protein is considered to be the most expensive component in animal feed, overfeeding thereof will also reduce the profit margins (NRC, 2007; Parisi *et al.*, 2020).

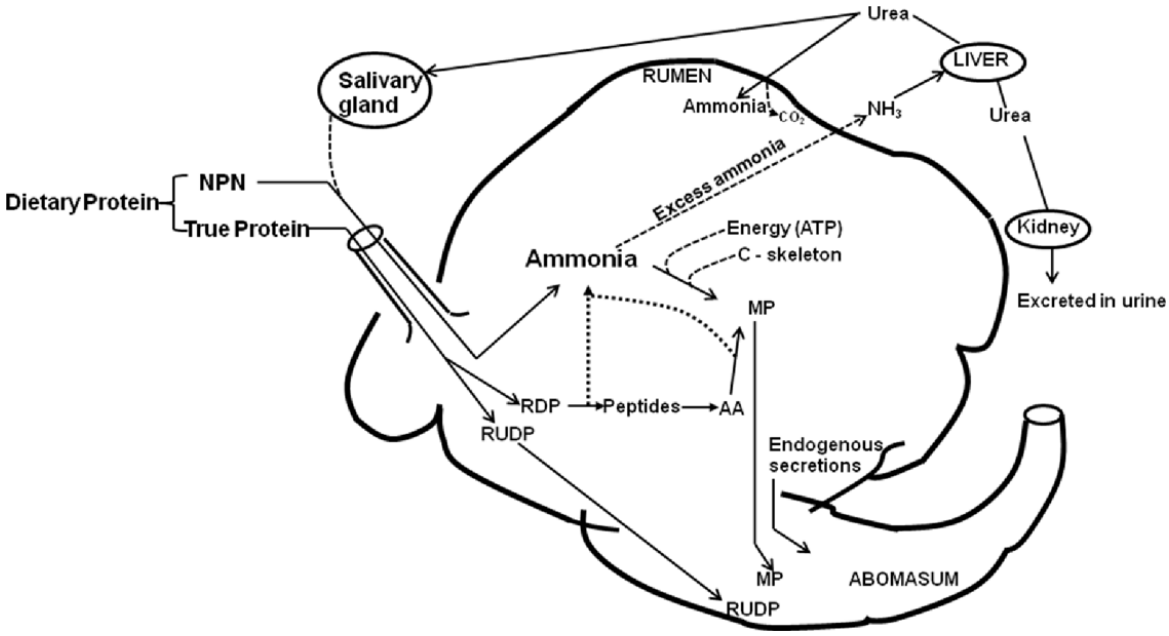


Figure 2.5 Overview of nitrogen metabolism in the rumen (Doranalli, 2010).

Strategies to use protein more efficiently in ruminant diets include feeding proteins with lower rumen degradability and, in some cases rumen-protected essential amino acids (EAA), which will lead to increasing levels of MP and EAA supplies, and therefore increase animal performance and reduce excreted nitrogen pollution (Broderick, 2018).

2.4. COMMONLY USED PROTEIN RAW MATERIALS IN SOUTH AFRICA

Oilseed meals are the residues that remain after most of the oil have been extracted from oilseeds, rendering high-quality protein feedstuffs. The two main processes in removing the oil from oilseeds involve either using pressure to force the oil out (expeller extracted), or using an organic solvent to dissolve oil from the seed (solvent extracted) (McDonald *et al.*, 2002). According to the AFMA raw material usage report of 2020, the oilseed meals mostly used in South Africa included soybean-, cotton-, sunflower- and canola meal.

Dried brewer's grain is a by-product of the beer industry that cannot be used directly for human consumption but can be included in animal diets (Jackowski *et al.*, 2020). In the sections below, the nutritional attributes and potential constraints of seven different protein feeds will briefly be discussed.

Table 2.3 Nutritional composition of different protein feeds (Adapted from NRC, 2007).

Feedstuff	DM	ME	CP	MP	RUP	CF	ADF	NDF	EE	Ash	Ca	P
	%	MJ/kg	%	%	%	%	%	%	%	%	%	%
Brewers Grains Dried	92	12.55	25	17.5	13.5	14	24	49	8.2	4	0.29	0.60
Canola Meal	90	10.87	40	28	12.0	12	20	29	4.0	8	0.75	1.16
Cottonseed Meal	92	12.13	46	32.2	23.0	13	18	31	5.0	7	0.21	1.19
DDGS (Maize)	90	13.8	29	20.3	14.5	9	17	43	10.6	6	0.28	0.79
Soybean Meal	91	12.55	49	34.3	17.2	6	10	15	1.6	7	0.38	0.71

All values except dry matter (DM) are shown on a dry matter basis. Energy expressions: metabolisable energy (ME). Protein expressions include crude protein (CP; Nitrogen x 6,25), metabolisable protein (MP), and rumen undegradable protein (RUP). Fibre expressions include crude fibre (CF), acid detergent fibre (ADF), and neutral detergent fibre (NDF). Ether extracts (EE) includes fatty acids, triglycerides, and other lipid compounds

2.4.1. **Soybean meal (SBM)**

Soybean meal is a by-product of oil extraction and is internationally the most important oilseed and livestock feed crop in the world, accounting for 58% of total world oilseed production and 69% of protein meal consumption by livestock (Board, 2012; Yildiz & Todorov, 2014; Stiles, 2016). It is a popular protein source used in livestock feed because of its palatability and high protein content (43-53%) (Yildiz & Todorov, 2014). It is further highly digestible and the amino acid profile is also very favourable (contains high amounts of lysine, tryptophan, threonine and isoleucine, which are often lacking in cereal grains) (McDonald *et al.*, 2002). A way to compare the protein quality of

different feed sources is to compare their lysine content percentage. The reason being is that lysine is usually considered the first limiting amino acid for animals (Yildiz & Todorov, 2014). Soy protein contains approximately 6.5%, lysine which is higher than many other oilseeds (canola meal protein is 5.8%, cottonseed meal – 4.2%, peanut meal – 3.4%, and sunflower meal –2.8%) (Yildiz & Todorov, 2014). However, due to the increasing cost and the negative environmental impact of production and distribution of SBM, there is a need to find alternative protein sources suitable to use in livestock feed. SBM also contain a few anti-nutritional factors that include trypsin inhibitors and lectins (McDonald *et al.*, 2002). These inhibitors are however deactivated with heat treatment during the processing of the soya (McDonald *et al.*, 2002).

2.4.2. **Canola meal (CM)**

Globally canola (rapeseed) meals are the second most used plant protein source used in animal feed (CCC, 2015). According to the industry statistics of AFMA (2020), in South Africa, CM is the 3rd most used oilcake in animal feed after soybean and sunflower meal. Canola is an offspring of rapeseed (*Brassica napus* and *Brassica campestris / rapa*), and with plant breeding techniques, the levels of the anti-nutritional components were lowered to < 2% of erucic acid in the oil portion and < 30 µmol/g of glucosinolates in the meal portion. Glucosinolates have a negative influence on palatability (Alexander *et al.*, 2008) and the degradation products of glucosinolates including isothiocyanates, thiocyanate, and nitriles are toxic to many livestock species (Lee *et al.*, 2020). Compared to soybean meal, canola co-products contain less crude protein (CP) and therefore less non-essential and essential amino acids, but greater ether extract (EE), acid detergent fibre (ADF), and neutral detergent fibre (NDF) (Lee *et al.*, 2020). The RUP (expressed as % of protein range between 40-56%, compared to 27-45% for SBM (CCC, 2015). Similar to many vegetable proteins, lysine is however limiting (McDonald *et al.*, 2002). Canola meal do contain high levels of methionine and cystine (McDonald *et al.*, 2002). Inclusion levels of CM in complete diets of sheep can be as high as 15% and 12% in the complete diets of dairy cows (PRF, 2018).

2.4.3. **Cottonseed meal (CSM)**

Cottonseed meal contains a crude protein content of 34.3%–48.9% (De Assis *et al.*, 2019). Relative to most other oilseeds CSM contains low levels of cystine, methionine and the first limiting amino acid lysine (McDonald *et al.*, 2002). Nutritional factors that influence the usage of CSM in livestock feed, include the presence of gossypol, a toxic phenolic compound (Gadelha *et al.*, 2014). Ruminants however are tolerant to gossypol and CSM can be used as a feedstuff in their ration (De Assis *et al.*, 2019). Cottonseed meal contains lower net energy, protein and lysine compared to SBM. Silva *et al.* (2016) reported that cottonseed meal can replace up to 100% of SBM without affecting

final bodyweight, daily weight gain, or total weight gain of feedlot lambs ($P > 0.05$). Anti-nutritional factors that influence the usage of CSM includes gossypol and non-nutritional factors includes availability and price (Williams & Ward, 1991). According to DAFF (2018b) the usage of CSM as a feed ingredient has reduced over the past three years in South Africa. The reasons is likely due to availability and price (DAFF, 2018b).

2.4.4. **Dried brewers grain (DBG)**

Brewers' grain is a by-product resulting from the production of beer and other malt products, it is the solid residue left after the processing of germinated and dried cereal grains (malt) (normally called brewer's spent grain) (Mussatto *et al.*, 2006). Although barley is the most common grain used to make beer, other grains can also be used like wheat, maize, rice, sorghum and millet (Dabija *et al.*, 2021). After the mashing process, most of the sugars have also been removed from the grain, the remaining brewery grains are high in protein and fibre that is particularly suitable for ruminants (Crawshaw, 2004). Being the most abundant brewing industry by-product (Ikram *et al.*, 2017), brewers grain may be a particularly cost-effective protein source because it is available at low cost from both large scale operations and small breweries (Mussatto *et al.*, 2006). A disadvantage of wet brewers grain is the poor shelf life, because of its high moisture content (70-81%) as well as the still present fermentable sugar contents (Santos *et al.*, 2003). This creates ideal conditions for microbial activity allowing rapid product deterioration (de Souza *et al.*, 2012). Preservation of wet brewery grain can be achieved by dehydrating as well as adding the advantage of reducing the product volume and therefore reducing the transport and storage cost (Mussatto *et al.*, 2006; Hatungimana, 2020). Another disadvantage of brewers grain is that the composition may vary which can be dependable on barley variety, time of harvest, characteristics of hops and other adjuncts added, and brewery technology (Santos *et al.*, 2003). Oven-dried brewers grain is a good feed for ruminants because it contains both high protein and fibre (Mussatto *et al.*, 2006). Brewer's grain is a lignocellulosic material rich in dietary fibre, which comprises of polysaccharides and lignin, that is resistant to hydrolysis by enzymes in the lower intestines (Ikram *et al.*, 2017). The nutrient composition of brewers grain consists of 15-26% crude protein, 3.9% crude fat and 3.4% ash DM, respectively (Santos *et al.*, 2003; Ikram *et al.*, 2017).

2.4.5. **Dried distillers' grains with solubles (DDGS)**

2.4.5.1. Background

Bioethanol is an alternative fuel source derived from plant-based biofuels (Pecka-Kielb *et al.*, 2017). Bioethanol production from sustainable biomass contributes to sustainability concerns of fossil fuel while also being more eco-friendly (Wang *et al.*, 2013).

The United States is the highest producer of biofuels, followed by Brazil, the production in South Africa is less than 0.01% (Pradhan & Mbohwa, 2014). Bioethanol and DDGS have exponentially increased since 1980 (Liu, 2011). This significant increase in production is illustrated in Figure 2.6.

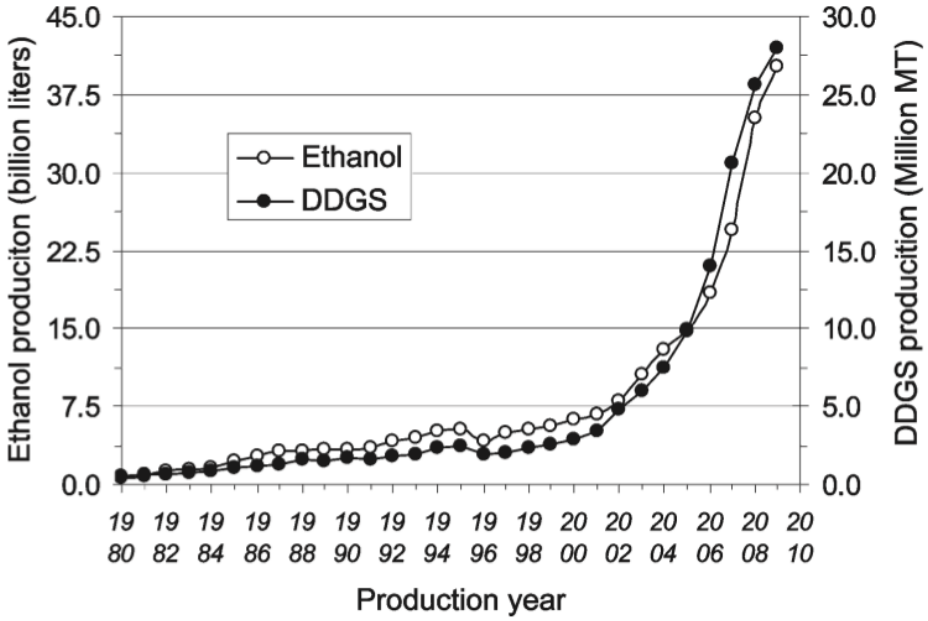


Figure 2.6 United States annual production of ethanol from grains and its main co-product, distillers dried grains with solubles (DDGS) between 1980 and 2010 (Liu, 2011).

Currently, South Africa is not producing biofuel on a large scale because of a restriction of the legislative process (Government Gazette, 2020). The reasons for this include concerns about impacts on food security, commodity prices and environmental and biodiversity concerns (Pradhan & Mbohwa, 2014).

Bioethanol, as an alternative to fossil fuels, is mainly produced by yeast fermentation from different carbohydrate sources: (i) sugar-containing raw materials: sugar beet, sugarcane, molasses, (ii) starch-containing feedstocks: grains such as maize and wheat, and (iii) lignocellulosic biomass: straw, agricultural waste, crop and wood residues (Bušić *et al.*, 2018b). The choice of crop used to produce bioethanol is linked to climatological factors, e.g., Central and South American countries

will use sugar cane, North America almost exclusively use maize and the EU will use cereals and maize and include crops such as sugar beets (Skoufogianni *et al.*, 2019).

It is a high-octane number fuel and its physicochemical features are noticeably different compared to gasoline (Bušić *et al.*, 2018a). Ethanol is produced with either wet or dry mill technology (Figure 2.7) (de la Piscina & Homs, 2008). These technological processes are similar but result in sightly different by-products. According to de la Piscina & Homs, (2008), dry grinding (Figure 2.8) is the primary method used for ethanol production (Pezzanite *et al.*, 2006) and provides a greater volume of bioethanol with the only byproduct of animal feed DDGS (if it is not dried wet distillers' grains with solubles, WDGS).

Considering quantity, the dry grind processing of 100 kg of maize produces roughly 40.2 L of ethanol, 32.3 kg of DDGS, and 32.3 kg of carbon dioxide (Schingoethe, 2006). Wet grind processing technology produces besides ethanol and animal feed other by-products like maize oil, maize syrup and gluten.

As shown in Figure 2.8, the first step in ethanol production using dry-grind technology is to reduce the particle size of maize by grinding it with a hammer mill. In the next step water and recycled stillage are added to the ground maize, which act as conditioners to begin leaching of soluble protein, sugars, and non-starch bound lipids (U.S. Grains Council, 2012). Cooking is then used to hydrolyze starch into glucose along with the addition of amylolytic enzymes in order for yeast (*Saccharomyces cerevisiae*) to convert glucose to ethanol (U.S. Grains Council, 2012). To produce ethanol a two-step fermentation of starch needs to take place (Kim *et al.*, 2008). The first step involves the hydrolysis of starch into glucose and maltose using two enzymes. In this stage (liquefaction) α-amylase is used, which results in dextrins end products (Kim *et al.*, 2008). Glucoamylase, during the saccharification stage, then converts dextrins into simple sugars, glucose and maltose (Kim *et al.*, 2008). In the next step, yeast fermentation (*Saccharomyces cerevisiae*) takes place, converting the obtained solution to ethanol and carbon dioxide. After fermentation, ethanol is recovered using distillation columns. During this step, the ethanol is still contaminated with water. Pure ethanol is then obtained with an ultrafiltration and pervaporation process with which the water is removed (U.S. Grains Council, 2012). The decoction that is left after distilling is separated into solid and liquid fractions with the use of decanters. The liquid fraction is then evaporated and condensed into syrup, which is mixed with the solid fraction from the decanters. The combination of these two fractions is centrifuged, dried and finally granulated resulting in the by-product DDGS (Pecka-Kielb *et al.*, 2017).

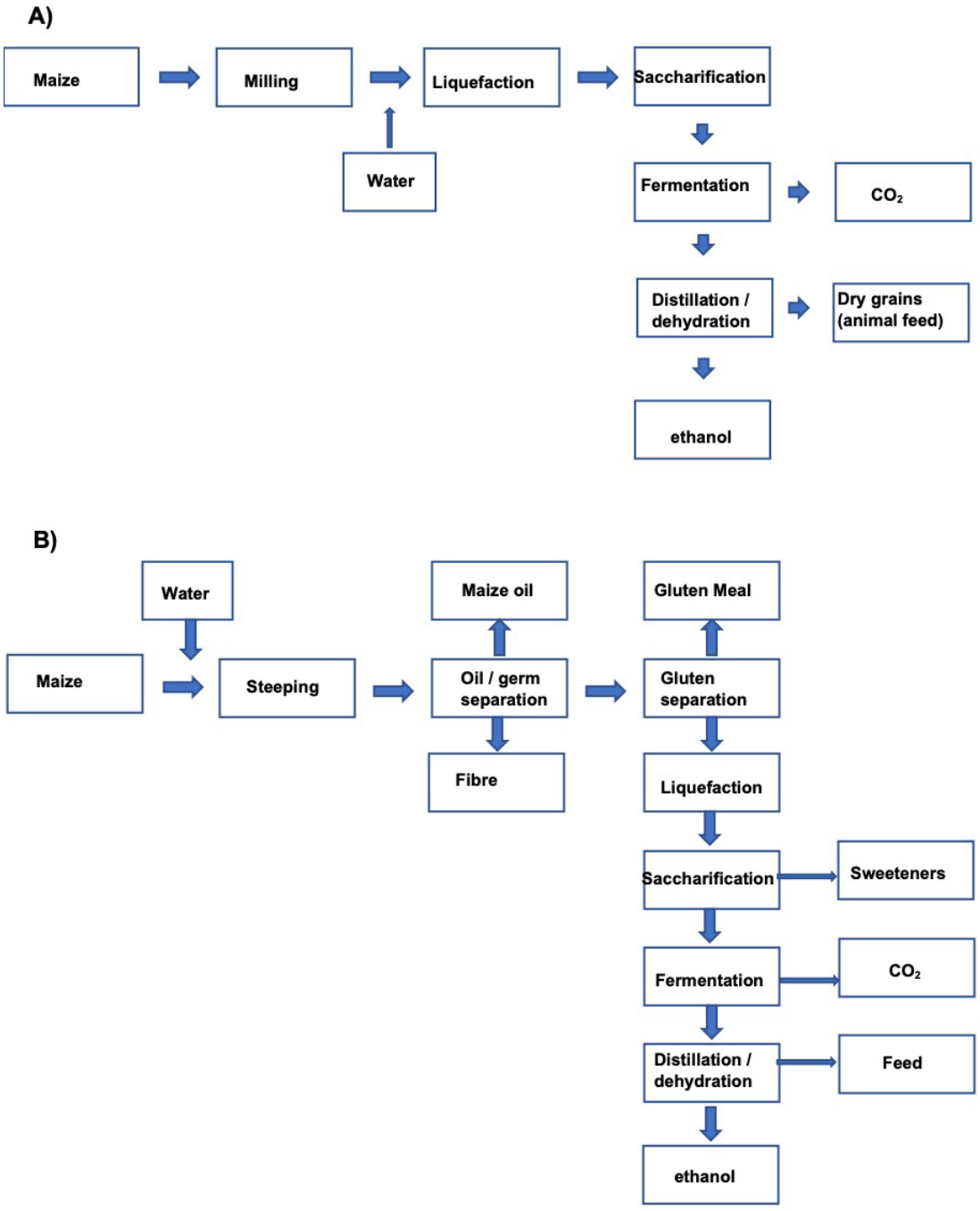


Figure 2.7 Ethanol production from maize: (A) dry mill process and (B) wet mill process (De la Piscina & Homs, 2008).

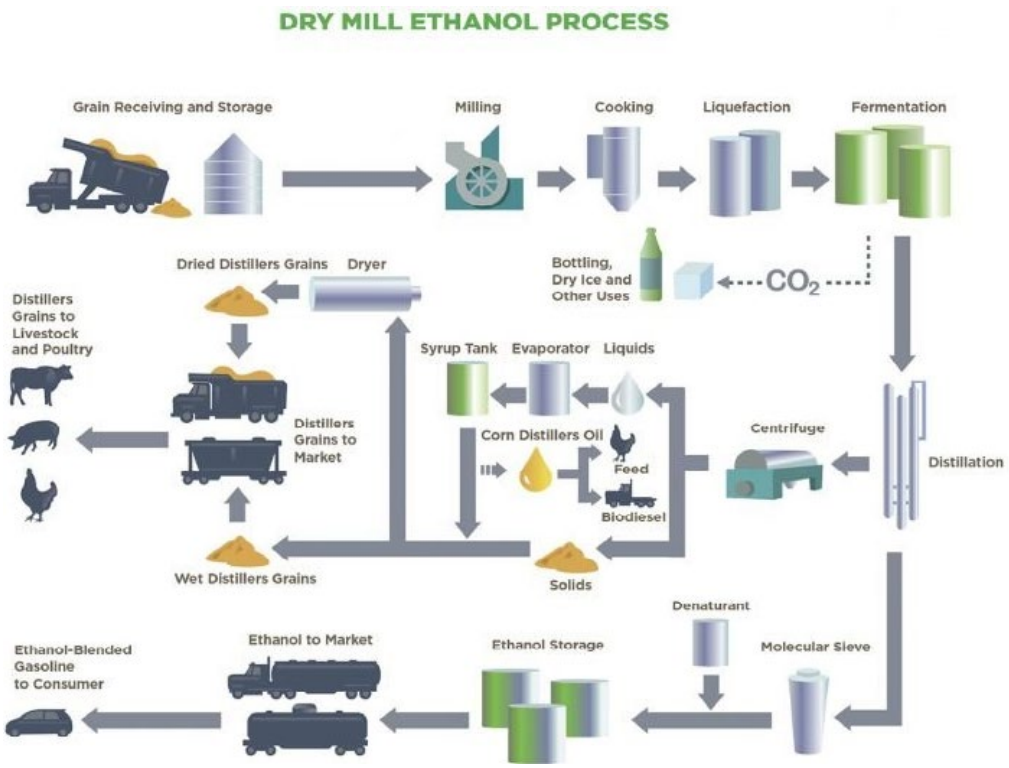


Figure 2.8 Entire dry milling process from delivery of maize to ethanol exiting the plant (Renewable Fuels Association, 2007).

The laws concerning the disposal of biofuel by-products became increasingly restrictive, while the use of DDGS as livestock feed is seen as sustainable (Popp *et al.,* 2016). The other benefit is, DDGS is normally also a more cost-effective alternative protein feedstuff in animal nutrition (Pecka-Kielb *et al.*, 2017). According to the grain analyst Mary Kennedy, (2020) the price of a ton DDGS in the USA (June 2020) was R 2359,80 per ton compared to SBM R4 955.58 per ton. The cost per unit of protein, therefore, results in R 87.38 and R 104.31 for DDGS (27% CP DM) and SBM (47.5% CP DM), respectively (Kennedy, 2020).

2.4.5.2. Nutritional characteristics of DDGS

Distillers grains have been used as an animal feed for more than 100 years (Loosli *et al.*, 1952), however, it is only recently that large quantities have become available and at competitive prices (Schingoethe *et al.*, 2009). Dried distillers grains with solubles derived from maize is an excellent source of energy and protein feed, containing 26-32% CP on a 100% dry matter basis (Liu, 2011). The protein of this by-product also has a low degradability rate in the rumen, which is beneficial in ruminant's feeding (Yildiz & Todorov, 2014) with a RUP fraction of 47% to 63% (average 55%) (Schingoethe, 2006). It is important to know the RUP and rumen degradable protein fractions of dietary protein when formulating diets (Yildiz & Todorov, 2014). Due to the drying regime, it causes

a negative reaction with some of the amino acids and carbohydrates, especially the first limiting amino acid (lysine), which make amino acids unavailable for absorption and metabolism (Yildiz & Todorov, 2014).

Dried distillers grains with solubles contain high amounts of insoluble fibre with an average NDF content of 38% (NRC, 2001) to 46% (NRC, 2000). The degradability of NDF in DDGS is quicker and higher compared to NDF in grains (Kononoff & Christensen, 2007). Zhang *et al.* (2010) partially replaced barley silage or barley grain with DDGS and evaluated the effect on DMI, milk yield and milk composition, chewing activity, and rumen fermentation of lactating dairy cows. The results of the latter authors show that the partial replacement of barley silage with DDGS, increased DMI and milk yield, milk protein, and lactose of lactating dairy cows. Although the dietary forage NDF of the DDGS was lower compared to the silage, no negative effects on rumen pH and rumen fermentation were observed (Zhang *et al.*, 2010). When barley grain was partially replaced with DDGS, rumen pH increased, without affecting milk yield (Zhang *et al.*, 2010). With a lower starch concentration of DDGS compared to grain (Liu, 2011), the risk of rumen acidosis is lowered and positively influences digestion. Furthermore, DDGS leads to a lower pathogen population and lessens the incidence of diarrhoea in young animals (Zhang *et al.*, 2010). Comparing the energy level of DDGS to other feed sources it contains a lower energy level than soya bean meal (by 4%), barley (by 17%), and wheat (by 25%), but higher than canola meal (20–40%) (Pecka-Kielb *et al.*, 2017). With soya bean meal being the most popular protein commodity used in animal nutrition, the average nutrient composition of SBM is compared to DDGS in Figure 2.9.

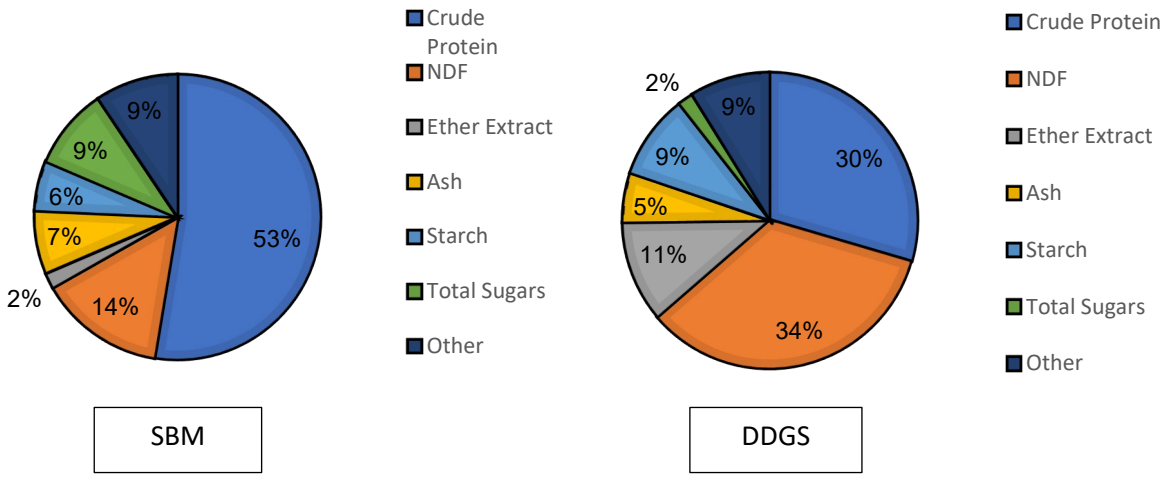


Figure 2.9 Nutrient composition of SBM and DDGS (Feedipedia, 2020).

2.4.5.3. Health concerns

Huls *et al.*, (2006) have concerns with the use of DDGS in finishing lamb diets because of the high phosphorous content and the potential environmental impact and possible problems with urinary calculi. Caution is further needed when formulating diets containing DDGS for sheep to prevent polioencephalomalacia due to the high sulphur (S) content of DDGS (Schauer *et al.*, 2008). This is due to excessive hydrogen sulphide gas production and lowered thiamine uptake in the rumen (Pezzanite *et al.*, 2006). Work at Purdue University demonstrated that supplementing 10mg of thiamine per 0.5kg of dietary intake, helped effectively to prevent polioencephalomalacia in lambs that consuming diets containing 25% to 50% DDGS (Pezzanite *et al.*, 2006). Normal recommendations from NRC (2007) are to keep dietary concentrations of S below 0.3 percent DM when animals are fed concentrate diets or below 0.5 percent DM when fed high-forage diets.

Historically, DDGS was not recommended to feed to ovine animals because of the high levels of copper. This was due to the distillation process that was conducted in copper stills primarily in traditional whisky distilleries (Cottrill *et al.*, 2007). In modern bioethanol production, however, distillation takes place in stainless steel stills, which eliminates the risk of high copper levels in DDGS and therefore the risk of copper poisoning to ovine animals. Dried distillers grains with solubles are also suspect to mycotoxin spoilage. Figure 2.10 shows the extent of mycotoxin concentrations in a 2011 survey study (Khatibi *et al.*, 2014) with a sample of 141 lots of 8 maize DDGS plants from 12 states in the USA.

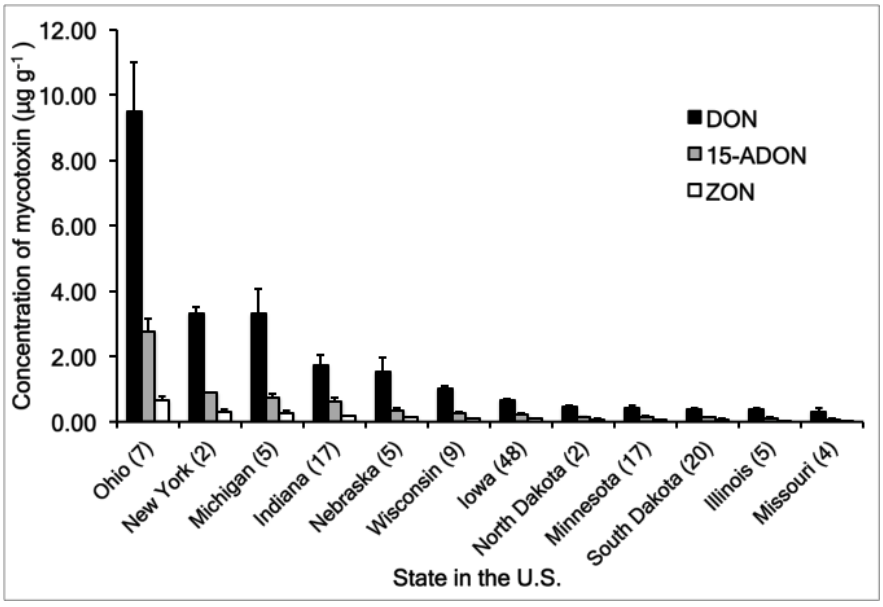


Figure 2.10 Mean concentrations (µg g−1) of DON, 15-ADON, and ZON for 141 lots of maize DDGS from 12 states in the U.S. in 2011(Khatibi *et al.*, 2014).

During the fermentation process, the mycotoxins are unfortunately not destroyed and therefore mycotoxins cannot be ignored (Pezzanite *et al.*, 2006). This is especially applicable if a mycotoxin problem existed in the original maize (Pezzanite *et al.*, 2006).

2.4.5.4. Variability

Ingredient consistency is critical to ensure optimal diet formulation and animal performance (Islam & Haque, 2016). As the ethanol production process is not standardized, it leads to variability in the by-products as well (de la Piscina & Homs, 2008). The inclusion of solubles, extraction (or not) of oil, and different innovations in fermentation and fractionation all result in DDGS that contains higher or lower protein, energy, fat, fibre and phosphorus levels. This variation often complicates accurately diet formulation with DDGS as an ingredient in practical environments (Schingoethe *et al.*, 2009). According to Cromwell *et al.* (1993), dark DDGS has a lower lysine digestibility than golden DDGS. Therefore the colour may be a quick and reliable indicator for the amino acid digestibility, especially of lysine, in DDGS (Böttger & Südekum, 2018).

2.4.5.5. Inclusion levels in feedlot lamb rations

Normally by-products as a protein source are not included at high levels in diets (Schauer *et al.*, 2008). Nevertheless, the increasing availability of biofuel by-products, together with sharp increases in maize commodity prices, makes DDGS a lucrative option to use as both a crude protein (CP) source and an energy substitute for maize (Van Emon *et al.*, 2012). Both Emon *et al.* (2012) and Shauer *et al,* (2008), suggest that lambs can be fed up to 50% DDGS without negative effects on feedlot performance. To evaluate what the inclusion rate could be before the point where sulphur (S) becomes potentially toxic, Schauer et al. (2008) included up to 60 percent DDGS to a lamb-finishing diet with no negative effects on feedlot performance or carcass traits. However, they did supplement thiamine to help with the prevention of S-induced polioencephalomalacia (Shauer *et al.,* 2008).

Felix *et al.* (2012), reported that ADG may be decreased at inclusion levels of higher than 60% DDGS. The latter authors also propose that at greater quantities of distillers grains the marbling score in lambs may be affected while a reduction in hot carcass weight (HCW) was also observed. According to these authors, the optimum dietary inclusion of DDGS for lambs is 20% of the DM. Increasing the dietary DDGS resulted in decreased digestion of DM and fat and the authors suggested that this could have been in part the reason for decreased lamb feedlot performance when 40 and 60% dietary DDGS was compared with 20% DDGS (Felix *et al.*, 2012).

2.5. CONCLUSION

Distiller's grains have been used for a long time as animal feed, however, in South Africa, it has not yet gained value as a feedstuff for sheep. Dried distillers grains with solubles may be a cost-effective feed option to use in the diet of lambs and can be used as either a protein or energy supplement. Especially with protein, which is the most expensive component of feed, and also a critical nutrient for young and growing animals. Traditionally soybean meal is used as the most reliant protein source in animal feed. The current price increases of SBM combined with a negative environmental production impact and pressure from consumers, forces feed manufacturers to investigate alternative sources of protein. Ruminant products will remain an important protein source to humans in the future. Ethical animal production using sustainable cost-effective ingredients to feed the animals will undoubtfully be a necessity in future. Dried distillers grains with solubles can to all likelihood make valuable contributions towards achieving this goal.

2.6. REFERENCES

Abraham, J. O., Hempson, G. P., & Staver, A. C. 2019. Drought-response strategies of savanna herbivores. Ecol. Evol. 9, 7047–7056. https://doi.org/10.1002/ece3.5270.

Abstract of Agricultural Statistics. 2019.in Directorate: Knowledge and Information Management. Pretoria, South Africa.

AFMA. 2020. AFMA Industry Statistics Archives. 2021. Available from: https://www.afma.co.za/download-category/afma-industry-statistics/.

Alexander, J., Auðunsson, G. A., Benford, D., Cockburn, A., Cravedi, J., Dogliotti, E., Domenico, A. Di, Férnandez-cruz, M. L., Fürst, P., Galli, C. L., Grandjean, P., Gzyl, J., Johansson, N., Mutti, A., Schlatter, J., Leeuwen, R. Van, & Van, C. 2008. Glucosinolates as undesirable substances in animal feed - Scientific Opinion of the Panel on Contaminants in the Food Chain. EFSA J. 6, 1–76. https://doi.org/10.2903/j.efsa.2008.590.

Anzai, H., Oishi, K., Kumagai, H., Hosoi, E., Nakanishi, Y., & Hirooka, H. 2017. Interspecific comparison of allometry between body weight and chest girth in domestic bovids. Sci. Rep. 7, 1–7.

Arnold, G. W., Wallace, S. R., & Maller, R. A. 1979. Some factors involved in natural weaning processes in sheep. Appl. Anim. Ethol. 5, 43–50. https://doi.org/10.1016/0304-3762(79)90006-3.

Atwood, S. B., Provenza, F. D., Wiedmeier, R. D., & Banner, R. E. 2001. Influence of free-choice vs mixed-ration diets on food intake and performance of fattening calves. J. Anim. Sci. 79, 3034–3040. DOI: 10.2527/2001.79123034x.

Bello, J. M., Mantecón, A. R., Rodriguez, M., Cuestas, R., Beltran, J. A., & Gonzalez, J. M. 2016. Fattening lamb nutrition. Approaches and strategies in feedlot. Small Rumin. Res. 142, 78–82. DOI : 10.1016/j.smallrumres.2016.04.006.

Berg, E. P., Neary, M. K., Thomas, D. L., & Kauffman, R. G. 1996. Assessment of Lamb Carcass Composition from Live Animal Measurement of Bioelectrical Impedance or Ultrasonic Tissue Depths. J. Anim. Sci., 2672–2678. DOI: 10.2527/1996.74112672x.

Board, J. E. (Ed). 2012. A Comprehensive Survey of international soybean - Research Genetics, Physiology, Agronomy and Nitrogen Relationships. InTech.

Böttger, C., & Südekum, K. H. 2018. Review: protein value of distillers dried grains with solubles (DDGS) in animal nutrition as affected by the ethanol production process. Anim. Feed Sci. Technol. 244, 11–17. https://doi.org/10.1016/j.anifeedsci.2018.07.018.

Bowen, M. K., Ryan, M. P., Jordan, D. J., Beretta, V., Kirby, R. M., Stockman, C., McIntyre, B. L., & Rowe, J. B. 2006. Improving sheep feedlot management. Pages 27–34 in Proceedings of the 2006 Australian Sheep Industry CRC Conference.

Brand, T. S., & Brundyn, L. 2015. Effect of supplementary feeding to ewes and suckling lambs on ewe and lamb live weights while grazing wheat stubble. South African J. Anim. Sci. 45, 89–95. https://doi.org/10.4314/sajas.v45i1.11.

Brand, T. S., & van der Merwe, G. D. 1993. Comparison of different protein sources in enriched grain mixture for fattening lambs. S. Afr. J. Anim. Sci. 23, 13–17. Available at: https://journals.co.za/doi/pdf/10.10520/EJC-1eb29164c1.

Brand, T. S., Van der Westhuizen, E. J., Van der Merwe, D. A., & Hoffman, L. C. 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(3), 477-488. http://dx.doi.org/10.4314/sajas.v48i3.8.

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86, 1370–1381. https://doi.org/10.3168/jds.S0022-0302(03)73721-7.

Broderick, G. A. 2018. Review: Optimizing ruminant conversion of feed protein to human food protein. Animal 12(8), 1722–1734. DOI: 10.1017/S1751731117002592.

Broderick, G. A., Wallace, R. J., Ørskov, E. R., & Hansen, L. 1988. Comparison of estimates of ruminal protein degradation by in vitro and in situ methods. J. Anim. Sci. 66, 1739–1745. https://doi.org/10.2527/jas1988.6671739x.

Brundyn, L., Brand, T. S., Ferreira, A. V, Aucamp, B. B., & Durand, A. 2008. The carry-over effect of supplementation in the previous year on the production of South African Mutton Merino ewes. South African J. Anim. Sci, 6, 19-24. Available at: https://www.sasas.co.za/wp-content/uploads/2012/10/Brundyn3CarryOverAPop05_0.pdf.

Bruwer, G.G., Grobler, I., Smit, M., Naudé, R.T., Vosloo, W.A., 1987. An evaluation ofthe lamb and mutton carcass grading system in the Republic of South Africa. 4. The influence of age, carcass mass and fatness on meat quality characteristics. South African J. Anim. Sci, 17, 95-103.

Bureau for Food and Agricultural Policy (BFAP). 2018. Baseline Agricultural outlook 2018-2027, University of Pretoria.

Bušić, A., Mardetko, N., Kundas, S., Morzak, G., Belskaya, H., Šantek, M. I., Komes, D., Novak, S., & Šantek, B. 2018a. Bioethanol production from renewable raw materials and its separation and purification: A review. Food Technol. Biotechnol. 56, 289–311. https://doi.org/10.17113/ftb.56.03.18.5546.

Bušić, A., Morzak, G., Belskaya, H., & Šantek, I. 2018b. Bioethanol Production from Renewable Raw Materials and Its Separation and Purification : A Review. Food Technol. Biotechnol. 56, 289–311. https://doi.org/10.17113/ftb.56.03.18.5546.

Calder, W. A. 1984. Size, Function and Life History. University Press, Cambridge, Massachusetts.

Campbell, B., Pullin, A., Pairis-Garcia, M., McCutcheon, J., Lowe, G., Campler, M., & Fluharty, F. 2017. The Effects of Alternative Weaning Strategies on Lamb Health and Performance. Small Rumin. Res. 56, 57–65. DOI : 10.1016/j.smallrumres.2017.09.006.

Cannas, A., Tedeschi, L. O., Atzori, A. S., & Lunesu, M. F. 2019. How can nutrition models increase the production efficiency of sheep and goat operations? Anim. Front. 9(2), 33–44. DOI: 10.1093/af/vfz005.

Casey, N. H., & Webb, E. C. 2010. Managing goat production for meat quality. Small Rumin. Res. 89, 218–224. https://doi.org/10.1016/j.smallrumres.2009.12.047.

CCC. 2015. Canola Meal Feeding Guide, 5th Edition, Canola Council of Canada.

Chiyangwa, T. 2018. Financial implications of converting from livestock to game in the karoo region, South Africa. MCom thesis. University of Stellenbosch, Stellenbosch, South Africa.

Cloete, S. W. P., & Olivier, J. J. 2010. South African Sheep and Wool Industries. Pages 95–112 in International Sheep and Wool Handbook.

Cloete, S. W. P., Snyman, M. A., & Herselman, M. J. 2000. Productive performance of Dorper sheep. Small Rumin. Res. 36, 119–135. https://doi.org/10.1016/S0921-4488(99)00156-X.

Colucci, P. E., MacLeod, G. K., Grovum, W. L., Cahill, L. W., & McMillan, I. 1989. Comparative Digestion in Sheep and Cattle Fed Different Forage to Concentrate Ratios at High and Low Intakes. J. Dairy Sci. 72, 1774–1785. https://doi.org/10.3168/jds.S0022-0302(89)79294-8.

Cottrill, B., Smith, C., Berry, P., Weightman, R., Wiseman, J., White, G., & Temple, M. 2007. Opportunities and implications of using the co- products from biofuel production as feeds for livestock. 99. Available at: https://www.researchgate.net/publication/241891535_Opportunities_and_Implications_of_Using_the_Co-products_from_Biofuel_Production_as_Feeds_for_Livestock/link/0f31752dfd3d4ed571000000/download.

Crawshaw, R. 2004. Co-product feeds: animal feeds from the food and drinks industries. Nottingham University Press.

Cromwell, G. L., Herkelman, K. L., & Stahly, T. S. 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. J Anim Sci. 71, 679–

686. https://doi.org/10.2527/1993.713679x. PMID: 8385086.

Dabija, A., Ciocan, M. E., Chetrariu, A., & Codină, G. G. 2021. Maize and sorghum as raw materials for brewing, a review. Appl. Sci. 11, 3139. https://doi.org/https://doi.org/10.3390/app11073139.

DAFF. 2018a. A Profile of the South African mutton market value chain. , 1–37.

DAFF. 2018b. South African Animal Feeds Market Analysis Report.

DAFF. 2019. Department of Agriculture, Forestry and Fisheries (DAFF). Pretoria.

DAFF. 2020. Trends in the Agricultural sector 2019. Pretoria.

Dally, M. & Hohenboken, W. 1979. Management Considerations for Accelerated Lambing in Western Oregon. Oregon State University Extension Service.

De Assis, D. Y. C., de Carvalho, G. P., Santos, E. M., de Oliveira, F. A., de Araujo, M. leonor G. M. L., Pina, D. dos S., Santos, S. A., & Rufino, L. M. de A. 2019. Cottonseed cake as a substitute of soybean meal for goat kids. Ital. J. Anim. Sci. 18, 124–133. https://doi.org/10.1080/1828051X.2018.1490633.

DeNicolo, G. 2007. Accelerated and out-of-season lamb production in New Zealand, PhD Thesis, Massey University, Palmerston North, New Zealand.

De la Piscina, P. ramirez, & Homs, N. 2008. Use of biofuels to produce hydrogen (reformation processes). Chem. Soc. Rev. 37, 2459–2467. https://doi.org/10.1039/b712181b.

Department of Agriculture. 2006. Agricultural production standards Act, 1990, Act number 119 regulation (R863). Natl. Gazettes, 1–20.

De Souza, L. C., Zambom, M. A., Pozza, M. S. dos S., Neres, M. A., Radis, A. C., Borsatti, L., Castagnara, D. D., & Gundt, S. 2012. Development of microorganisms during storage of wet brewery waste under aerobic and anaerobic conditions. Rev. Bras. Zootec. 41, 188–193. https://doi.org/10.1590/S1516-35982012000100027.

Doranalli, K. 2010. Factors Regulating Urea-Nitrogen Recycling in Ruminants.. PhD thesis, University of Saskatchewan, Canada.

Duddy, G., Shands, C., Bell, A., Hegarty, R., & Casburn, G. 2016. Feedlotting lambs. NSW Department of Primary Industries.

Erickson, P. S., & Kalscheur, K. F. 2020. Nutrition and feeding of dairy cattle. Anim. Agric. Sustain. Challenges Innov., 157–180. https://doi.org/10.1016/B978-0-12-817052-6.00009-4.

Feedipedia. 2020. Soybean meal. [Internet document] Available from URL: http://https://www.feedipedia.org/node/674 (Accessed 8 July 2020).

Felix, T. L., Zerby, H. N., Moeller, S. J., & Loerch, S. C. 2012. Effects of increasing dried distillers grains with solubles on performance, carcass characteristics, and digestibility of feedlot lambs. J. Anim. Sci. 90, 1356–1363. https://doi.org/10.2527/jas.2011-4373.

Forbes, B. Y. J. M., & Kyriazakis, I. 1995. Food preferences in farm animals : why don't they always choose wisely ? Proc. Nutr. Soc. 54(2), 429-40. DOI: 10.1079/pns19950012.

Gadelha, I. C. N., Fonseca, N. B. S., Oloris, S. C. S., Melo, M. M., & Soto-Blanco, B. 2014. Gossypol toxicity from cottonseed products. Sci. World J. 2014, 231635. https://doi.org/10.1155/2014/231635.

Government Notice No. R. 863 2006. Agricultural product standards Act 119 of1990. Regulations regarding the classification and marketing ofmeat in the Republic ofSouth Africa.

Government Gazette. 2020. Energy Mineral Resources: South African Biofuels Regulatory Framework and National Biofuels Feedstock Protocol. Department mineral resources and energy. Vol 656, No. 43003. Pretoria, RSA.

http://www.energy.gov.za/files/policies/petroleum/Biofuels-Regulato.

Hackmann, T. J., & Spain, J. N. 2010. Invited review: Ruminant ecology and evolution: Perspectives useful to ruminant livestock research and production. J. Dairy Sci. 93, 1320–1334. https://doi.org/10.3168/jds.2009-2071.

Hatungimana, E. 2020. University of New Hampshire Scholars ' Repository Preservation and Utilization of Wet Brewer's Grains in Diets of Dairy Heifers. PhD thesis, University of New Hampshire, Durhan, USA.

Henchion, M., McCarthy, M., Resconi, V. C., & Troy, D. 2014. Meat consumption: Trends and quality matters. Meat Sci. 98, 561–568. https://doi.org/10.1016/j.meatsci.2014.06.007.

Hernández, J., Benedito, J. L., Abuelo, A., & Castillo, C. 2014. Ruminal acidosis in feedlot: From aetiology to prevention. Sci. World J. 2014, Article ID 702572. https://doi.org/10.1155/2014/702572.

Hofmann, A. R. R. 1989. International Association for Ecology Evolutionary Steps of Ecophysiological Adaptation and Diversification of Ruminants : A Comparative View of Their Digestive System Published by : Springer in cooperation with International Association for Ecology Stabl. Oecologia 78(4), 443–457. DOI: 10.1007/BF00378733.

Hoskin, G. 2015. https://www.timeslive.co.za/news/south-africa/2015-01-14-foot-and-mouth-eats-up-farming/. Sunday Times.

Huls, T. J., Bartosh, A. J., Daniel, J. A., Zelinsky, R. D., Held, J., & Wertz-Lutz, A. E. 2006. Efficacy of Dried Distiller's Grains with Solubles as a Replacement for Soybean Meal and a Portion of

the Corn in a Finishing Lamb Diet 1. Sheep Goat Res. J. 21. Available at: https://www.researchgate.net/publication/251446815_Efficacy_of_Dried_Distiller's_Grains_with_Solubles_as_a_Replacement_for_Soybean_Meal_and_a_Portion_of_the_Corn_in_a_Finishing_Lamb_Diet1/link/0a85e53284741a5639000000/download.

Ikram, S., Huang, L. Y., Zhang, H., Wang, J., & Yin, M. 2017. Composition and Nutrient Value Proposition of Brewers Spent Grain. J. Food Sci. 82, 2232–2242. https://doi.org/10.1111/1750-3841.13794.

Islam, M. S., & Haque, M. M. 2016. Quality Control of Raw Materials for Poultry Feed. J. Poult. Sci. Technol. 4, 19–27.

Jackowski, M., Niedźwiecki, Ł., Jagiełło, K., Uchańska, O., & Trusek, A. 2020. Brewer's spent grains—valuable beer industry by-product. Biomolecules 10, 1–18. https://doi.org/10.3390/biom10121669.

Janvosky, E. 2016. Wildlife industry expected to grow. Wildl. Breeders J., 12–14. Availabe at: http://www.redmeatsa.co.za/wp-content/uploads/2015/07/Game-Outlook.pdf.

Jayawardena, V. P. 2000. Observations on the Ruminal protein degradation products and the absorption of ruminally derived free and peptidebound amino acids via ovine forestomach epithelia in vitro. PhD thesis, Virginia Polytechnic Institute and State University, USA.

Jolly, S., & Wallace, A. 2007. Best practice for production feeding of lambs: a review of the literature. Meat & Livestock Australia Limited.

Kennedy, M. 2020. Distiller's Grains: Under Pressure as Feeders Look for Cheaper Prices – DTN. https://agfax.com/2020/06/05/distillers-grains-under-pressure-as-feeders-look-for-cheaper-prices-dtn/.

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

Khatibi, P. A., McMaster, N. J., Musser, R., & Schmale, D. G. 2014. Survey of mycotoxins in corn distillers' dried grains with solubles from seventy-eight ethanol plants in Twelve states in the U.S. in 2011. Toxins (Basel). 6, 1155–1168. https://doi.org/10.3390/toxins6041155.

Kim, Y., Mosier, N. S., Hendrickson, R., Ezeji, T., Blaschek, H., Dien, B., Cotta, M., Dale, B., & Ladisch, M. R. 2008. Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. Bioresour. Technol. 99, 5165–5176. https://doi.org/10.1016/j.biortech.2007.09.028.

Kononoff, P. J., & Christensen, D. A. 2007. Feeding dried distillers grain to dairy cattle. Western

Nutritional Conference (USA), pp. 1-13.

Landman, A. M. 2013. Economic analysis of intensive sheep production systems in central South Africa. MSc thesis, University of the Free State, Bloemfontein, South Africa.

Larson, R. C. 2017. Economic analysis of profitability factors in cattle feeding: Modeling optimal feeding to achieve maximum profitabilty. MSc Thesis, Colorado State University, Fort Collins, Colorado, USA.

Lee, J. W., Kim, I. H., & Woyengo, T. A. 2020. Toxicity of Canola-Derived Glucosinolate Degradation Products in Pigs — A Review. 10(2), 2337. doi: 10.3390/ani10122337.

Lewis, R. M., Notter, D. R., Hogue, D. E., & Magee, B. H. 1996. Ewe fertility in the STAR accelerated lambing system. J. Anim. Sci. 74, 1511–1522. https://doi.org/https://doi.org/10.2527/1996.7471511x.

Li, B., Sun, X., Huo, Q., Zhang, G., Wu, T., You, P., He, Y., Tian, W., Li, R., Li, C., Li, J., Wang, C., & Song, B. 2021. Pelleting of a Total Mixed Ration Affects Growth Performance of Fattening Lambs. Front. Vet. Sci. 8, 1–13. https://doi.org/10.3389/fvets.2021.629016.

Lima, N. L. L., de Freitas Ribeiro, C. R. R., de Sa, H. C. M., Leopoldino-Júnior, I., Cavalcanti, L. F. L., Santana, R. A. V., Furusho-Garcia, I. F., & Pereira, I. G. 2017. Economic analysis, performance, and feed efficiency in feedlot lambs. Rev. Bras. Zootec. 46, 821–829. https://doi.org/10.1590/S1806-92902017001000005.

Liu, K. 2011. Chemical Composition of Distillers Grains, a Review. J. Agric. Food Chem. 59, 1508–1526. https://doi.org/dx.doi.org/10.1021/jf103512z |.

Loor, J. J., Elolimy, A. A., & McCann, J. C. 2016. Dietary impacts on rumen microbiota in beef and dairy production. Anim. Front. 6, 22–29. https://doi.org/10.2527/af.2016-0030.

Loosli, J. K., Turk, K. L., & Morrison, F. B. 1952. THE VALUE OF DISTILLERS FEEDS. J. Dairy Sci. 35, 868–873. https://doi.org/10.3168/jds.S0022-0302(52)93768-5.

Louw, D., Louw, A., & Flandorp, C. 2018. A Study on the potential product development for the commercialization and value add to beef products, OABS Development (Pty) Ltd. Paarl, South Africa.

Male, J. 2012. Lamb Finishing Systems. Nuffield Australia Project No 1115, MLA. Available at: https://www.nuffieldscholar.org/sites/default/files/reports/2011_AU_James-Male_Lamb-Finishing-Systems-Maximising-The-Margins-On-Grain-Finishing-Lambs.pdf.

Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., Lucchin, L., & Poli, A. 2015. Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. Food Nutr. Res. 59, 27606. DOI: 10.3402/fnr.v59.27606.

Mare, F., Bahta, Y. T., & Van Niekerk, W. 2018. The impact of drought on commercial livestock farmers in South Africa. Dev. Pract. 28, 884–898. https://doi.org/10.1080/09614524.2018.1493091.

McDonald, P., Edwards, R., Edwards, R. A., Greenhalgh, J. F. D., & Morgan, C. A. 2002. Animal Nutrition. 6th ed. Pearson, Essex, England.

Mcdonald, C. L., Gittins, S. P., & Rowe, J. B. 1988. Effect of time of year and prior feeding experience on feeding behaviour of sheep as if for live export. Proc. Aust. Soc. Anim. Prod. 17, 226–229.

Meissner, H. H., Hofmeyr, H. F., & van Rensburg, W.J.J.J. and Pienaar, J. P. 1983. Classification of livestock for realistic prediction of substitution values in terms of a biologically defined Large Stock Unit.in Technical Communication No. 175. Department of Agriculture, Pretoria, South Africa.

Meissner, H. H., Scholtz, M. M., & Engelbrecht, F. A. 2013. Sustainability of the South African livestock sector towards 2050 part 2: Challenges, changes and required implementations. S. Afr. J. Anim. Sci. 43, 298–319. DOI:10.4314/SAJAS.V43I3.6.

Mercer, J. R., Allen, S. A., & Miller, E. L. 1980. Rumen bacterial protein synthesis and the proportion of dietary protein escaping degradation in the rumen of sheep. Br. J. Nutr. 43(3), 421–433. DOI: 10.1079/bjn19800110.

MLA. 2007. A producers' guide to production feeding for lamb growth. Meat & Livestock Australia Limited.

Mussatto, S. I., Dragone, G., & Roberto, I. C. 2006. Brewers' spent grain: Generation, characteristics and potential applications. J. Cereal Sci. 43, 1–14. https://doi.org/10.1016/j.jcs.2005.06.001.

NRC, 1984. Nutrient requirements of beef cattle. 6th ed. The National Academies, Washington, D.C.

NRC, 1996. Nutrient requirements of beef cattle. 7th ed. The National Academies, Washington, D.C.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. The National Academies Press, Washington, DC.

NRC. 2001. Nutrient Requirements of Dairy Cattle. seventh. The National Academies Press, Washington DC.

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

Owens, F. N., Dubeski, P., & Hanson, C. F. 1993. Factors that alter the growth and development of ruminants. J. Anim. Sci. 71, 3138–3150. DOI: 10.2527/1993.71113138x.

Parisi, G., Tulli, F., Fortina, R., Marino, R., Bani, P., Dalle Zotte, A., De Angeli, A., Piccolo, G., Pinotti,

L., Schiavone, A., Terova, G., Prandini, A., Gasco, L., Roncarati, A., & Danieli, P. P. 2020. Protein hunger of the feed sector: the alternatives offered by the plant world. Ital. J. Anim. Sci. 19, 1205–1227. https://doi.org/10.1080/1828051X.2020.1827993.

Pasmans, T., & Hebinck, P. 2017. Rural development and the role of game farming in the Eastern Cape, South Africa. Land use policy 64, 440–450. https://doi.org/10.1016/j.landusepol.2017.03.010.

Pecka-Kielb, E., Zachwieja, A., Mista, D., Zawadzki, W., & Zielak-Steciwko, A. 2017. Use of Corn Dried Distillers Grains (DDGS) in Feeding of Ruminants. DOI:10.5772/66357.

Pérez-Barbería, F. J. 2020. The Ruminant: Life History and Digestive Physiology of a Symbiotic Animal. In: Sustainable and Environmentally Friendly Dairy Farms. Springer Briefs in Applied Sciences and Technology. Springer, Cham. https://doi.org/https://doi.org/10.1007/978-3-030-46060-0_2.

Peters, R. H. 1983. The Ecological Implications of Body Size. Cambridge University Press.

Pezzanite, L., Student, A. S., Gunn, P., Neary, M., Small, E., Specialist, R., Lemenager, R., Specialist, E. B., Lake, S., & Specialist, E. B. 2006. Value of distiller's grains as a feed for sheep. Purdue extention paper AS-603-W. Available at: https://www.extension.purdue.edu/extmedia/AS/AS-603-W.pdf.

Phakathi, B. 2020. https://www.businesslive.co.za/bd/national/2020-01-13-dire-prediction-for-sa-beef-exports-after-foot-and-mouth-outbreak/. Business Day.

Pienaar, N. J., & Thompson, P. N. 2013. Temporal and spatial history of Rift Valley fever in South Africa : 1950 to 2011. Onderstepoort J. Vet. Res. 80(1), 1–13. DOI: 10.4102/ojvr.v80i1.384.

Povey, G., Stubbings, L., & Phillips, K. 2016. Feeding the Ewe A Literature Review. Available at: https://projectblue.blob.core.windows.net/media/Default/Research%20Papers/Beef%20&%20Lamb/Feeding-the-Ewe-–-Review-Final-Version-050916.pdf.

Pradhan, A., & Mbohwa, C. 2014. Development of biofuels in South Africa: Challenges and opportunities. Renew. Sustain. Energy Rev. 39, 1089–1100. https://doi.org/10.1016/j.rser.2014.07.131.

PRF. 2018. Canola Production Guide. 3rd ed. Protein Research Foundation, Rivonia.

Raoult, C. M. C., Osthaus, B., Hildebrand, A. C. G., McElligott, A. G., & Nawroth, C. 2021. Goats show higher behavioural flexibility than sheep in a spatial detour task. R. Soc. Open Sci. 8, 201627. https://doi.org/10.1098/rsos.201627.

Renewable Fuels Association. 2007. "U.S. fuel ethanol industries biorefineries and production capacities." Accessed November 29, 2021 from

<http://www.ethanolrfa.org/industry/locations/>.

Rice, M., Jongman, E. C., Borg, S., Butler, K. L., & Hemsworth, P. H. 2016. Characterisation of Shy-feeding and Feeding lambs in the first week in a feedlot. Appl. Anim. Behav. Sci. 179, 39–45. DOI : 10.1016/j.applanim.2016.03.011.

Rodríguez, A. B., Bodas, R., Fernández, B., López-Campos, O., Mantecón, A. R., & Giráldez, F. J. 2007. Feed intake and performance of growing lambs raised on concentrate-based diets under cafeteria feeding systems. Animal 1, 459–466. https://doi.org/10.1017/S1751731107683803.

Rowe, J. B., Choct, M., & Pethick, D. W. 1999. Processing cereal grains for animal feeding. Aust. J. Agric. Res. 50, 721–736. https://doi.org/10.1071/AR98163.

Rusche, W. 2020. Adding value to corn through cattle. South Dakota State University Extension paper, Brookings, USA. Avalable at: https://extension.sdstate.edu/adding-value-corn-through-cattle.

Ruzic-Muslic, D., Petrovic, M. P., Petrovic, M. M., Bijelic, Z., Caro-Petrovic, V., Maksimovic, N., & Mandic, V. 2014. Protein source in diets for ruminant nutrition. Biotechnol. Anim. HusbandryBiotehnologija u Stoc. 30, 175–184. https://doi.org/10.2298/bah1402175r.

Santos, M., Jiménez, J. J., Bartolomé, B., Gómez-Cordovés, C., & Del Nozal, M. J. 2003. Variability of brewer's spent grain within a brewery. Food Chem. 80, 17–21. https://doi.org/10.1016/S0308-8146(02)00229-7.

Schauer, C. S., Stamm, M. M., Maddock, T. D., & Berg, P. B. 2008. Feeding of DDGS in lamb rations. Sheep Goat Res. J. 23, 15–19. Awailable at: https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/ba3468a2a8681f69872569d60073fde1/5703a371897ed680872575d200764f58/$FILE/Feeding_of_DDGS_in_lamb_rations.pdf.

Schingoethe, D. J. 2006. Utilization of DDGS by Cattle.in 27th Western Nutrition Conf., Winnipeg, Manitoba, Canada.

Schingoethe, D. J., Kalscheur, K. F., Hippen, A. R., & Garcia, A. D. 2009. Invited review : The use of distillers products in dairy cattle diets. J. Dairy Sci. 92, 5802–5813. https://doi.org/10.3168/jds.2009-2549.

Schwab, C. G., Tylutki, T. P., Ordway, R. S., Sheaffer, C., & Stern, M. D. 2003. Characterization of proteins in feeds. J. Dairy Sci. 86, E88–E103. https://doi.org/10.3168/jds.S0022-0302(03)74042-9.

Sheridan, R., Ferreira, A. V, & Hoffman, L. C. 2003. Production efficiency of South African Mutton Merino lambs and Boer goat kids receiving either a low or a high energy feedlot diet. Small Rumin. Res. 50, 75–82. https://doi.org/10.1016/S0921-4488(03)00109-3.

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 Araújo, M. L. G. M. L., de Oliveira Nascimento, C., Santos, S. A., Tosto, M. S. L., de Almeida Rufinoa, L. M., & de Carvalho, B. M. A. 2016. Cottonseed cake in substitution of soybean meal in diets for finishing lambs. Small Rumin. Res. 137, 183–188. https://doi.org/10.1016/j.smallrumres.2016.03.014.

Simões, J., Abecia, J. A., Cannas, A., Delgadillo, J. A., Lacasta, D., Voigt, K., & Chemineau, P. 2021. Review: Managing sheep and goats for sustainable high yield production. Int. J. Anim. Biosci. Suppl 1, 100293. https://doi.org/10.1016/j.animal.2021.100293.

Skoufogianni, E., Solomou, A., Charvalas, G., & Danalatos, N. 2019. Maize as Energy Crop, Maize - Production and Use Maize. IntechOpen, DOI: 10.5772/intechopen.88969.

Stanton, T., & LeValley, S. 2006. Lamb feedlot nutrition, Colorado State University Extension, Fact Sheet No. 1.613. https://extension.colostate.edu/docs/pubs/livestk/01613.pdf.

Stiles, W. 2016. The case for alternative protein sources for animal feed. Available at: https://businesswales.gov.wales/farmingconnect/sites/farming/files/technical_article_-_protein_alternatives_final_1.pdf.

Terblanche, S. 2013. An economic analysis of an intensive sheep production system on cultivated pastures in South Africa, incorporating creep feeding- and accelerated breeding practices. MTech Thesis, Nelson Mandela Metropolitan University.

Toldra, F. (Ed). 2017. Lawrie's Meat Science. 8th ed. Woodhead Publishing.

U.S. Grains Council. 2012. A guide to distiller's dried grains with solubles (DDGS). 3rd ed.https://doi.org/10.1079/bjn19800110.

Van der Merwe, D. A., Brand, T. S., & Hoffman, L. C. 2020. Premium lamb production of different South African sheep breed types under feedlot conditions. S. Afr. J. Anim. Sci., 1–12. doi.org/10.4314/sajas.v50i4.9.

Van der Merwe, D. A., 2020. Modelling the growth, feed intake and backfat deposition of different South African sheep breed types. Thesis (PhDAgric). University of Stellenbosch, South Africa.

Van Emon, M. L., Gunn, P. J., Neary, M. K., Lemenager, R. P., Schultz, A. F., & Lake, S. L. 2012. Effects of added protein and dietary fat on lamb performance and carcass characteristics when fed differing levels of dried distiller's grains with solubles. Small Rumin. Res. 103, 164–168. https://doi.org/10.1016/j.smallrumres.2011.09.002.

Van Niekerk, N. 2020. Personal communication, Caledon, South Africa.

Van Wyk, D. N. 2011. A quantitative analysis of supply response in the Namibian mutton industry. MSc (Agric) thesis. University of Stellenbosch, South Africa.

Wang, L., Littlewood, J., & Murphy, R. J. 2013. Environmental sustainability of bioethanol production from wheat straw in the UK. Renewable and Sustainable Energy Reviews 28., 715–725. https://doi.org/https://doi.org/10.1016/j.rser.2013.08.031.

Webb, E. C., & Erasmus, L. J. 2013. The effect of production system and management practices on the quality of meat products from ruminant livestock. South African J. Anim. Sci. 43, 415–423. http://dx.doi.org/10.4314/sajas.v43i3.11.

Webb, E. C. 2015. Description of carcass classification goals and the current situation in South Africa. South African J. Anim. Sci. 45, 229-233.

Wenk, C., Pfirter, H. P., & Bickel, H. 1980. Energetic aspects of feed conversion in growing pigs, Livestock Production Science. Livest. Prod. Sci. 7, 483–495. https://doi.org/10.1016/0301-6226(80)90086-X.

Williams, G. W., & Ward, J. B. 1991. The Use of Cottonseed Meal in Animal feed rations : A survey of feed formulators. Agribiz. 10(2), 115-130. DOI: 10.1002/1520-6297(199403/04)10:2<115::AID-AGR2720100203>3.0.CO;2-I.

Yildiz, E., & Todorov, N. 2014. The comparison of the main protein sources for dairy cows. A review. Bulg. J. Agric. Sci. 20, 428–446. Available at: https://kipdf.com/the-comparison-of-the-main-protein-sources-for-dairy-cows-a-review_5ab093b41723dd329c635e9a.html.

Zhang, S. Z., Penner, G. B., Yang, W. Z., & Oba, M. 2010. Effects of partially replacing barley silage or barley grain with dried distillers grains with solubles on rumen fermentation and milk production of lactating dairy cows. J. Dairy Sci. 93, 3231–3242. https://doi.org/10.3168/jds.2009-3005.

CHAPTER 3

AN *IN VITRO* EVALUATION OF RUMEN KINETICS OF SOYBEAN MEAL, COTTONSEED MEAL, DRIED BREWERS GRAIN, CANOLA MEAL AND DRIED DISTILLERS' GRAINS WITH SOLUBLES.

3.1. ABSTRACT

The objective of this study was to compare the extent and rate of ruminal protein degradation of different protein sources against dried distillers' grains with solubles for ruminants. Five different protein samples, soybean meal (SBM), cottonseed meal (CSM), dried brewers grain (DBG), canola meal (CM) and distillers' grains with solubles (DDGS) were incubated in buffered ruminal fluid for 0, 2, 4, 8, 16, 24, and 48 h, respectively. The effective degradability was calculated using k_p = 0.05 and 0.08. Estimated effective CP degradability coefficients (k_p = 0.05/h) did not differ significantly between SBM and CM (P > 0.05). However, differences for both SBM and CM were observed among CSM, DBG and DDGS (P < 0.05). The estimated effective CP degradability coefficients (k_p = 0.05/h) for SBM, CSM, DBG, CM and DDGS were 0.60, 0.40, 0.30, 0.57, and 0.55, respectively. The observed rumen undegradable protein (RUP) content (% of CP) with a ruminal passage rate of 0.08/h were as follows: SBM (47%) < CM (49%) < DDGS (50%) < cottonseed oilcake (64%) < DBG (68%), respectively. No differences (P > 0.05) were observed in the extent of ruminal degradation between soya bean meal and DDGS with a ruminal passage rate of 0.08/h. The kinetic parameters and estimated effective CP degradability were affected by the feed sources evaluated.

3.2. Introduction

Protein supplements are expensive and represent a large portion of ruminant diets (Huuskonen *et al.*, 2014). It is, therefore, an important consideration for economic success in the livestock industry (Yildiz & Todorov, 2014). According to Ruzic-Muslic *et al.* (2014), protein sources have a significant influence on the ultimate/final result of fattened lambs, especially if the protein bypasses the reticulum-rumen undegraded, in the presence of sufficient energy. In Chapter 2, the types of crude protein (CP) the ruminant can obtain from feed have been discussed, as well as the role each one plays in ruminant nutrition. It has long been documented that the degradation rate in the rumen is required to determine the protein value of feedstuffs for ruminants (Ørskov & Mcdonald, 1979). By solely using dietary CP to formulate diets and not taking the ruminal degradation characteristics into account, might result in inefficient use of protein (Ørskov & Mcdonald, 1979; Das *et al.*, 2015). Due to the activities of ruminal microorganisms such as degradation of feed protein,

synthesis of microbial protein and passage of both undegraded feed protein and microbial protein to lower intestine is why CP values exclusively cannot be used accurately in ruminant diet formulation (Das *et al*, 2014). Therefore protein analysis and degradation rates are required to accurately formulate balanced diets (Pacheco *et al.*, 2012). Although soybean meal (SBM) is the preferred source of protein for animals in South Africa (DAFF, 2020), dried distiller's grains with solubles (DDGS) and brewers' spent grains are sustainable protein sources for ruminant feeds due to availability, economic and environmental concerns. According to Liu *et al.* (2019), these by-products can provide competitive alternatives to more traditional protein sources. Data for rumen degradability of DDGS in South Africa are, however, limited.

Methods that are used to evaluate the protein degradability or digestion include *in vivo, in situ* and *in vitro* (Cömert Acar, 2018). The conventional *in vivo* method is the most reliable and accurately reflects the feed value and degradability of the protein source used in the total diet, however, it is labour intensive, time-consuming and expensive (López, 2009). Several methods have been developed to replicate the *in vivo* methods to determine rumen undegradable protein (RUP) digestibility.

The *in situ* or *in sacco* (bag technique) is a well-known, simple and dependable method to estimate the degradability of dry matter (DM) and protein in the rumen (Mohamed & Chaudhry, 2017). This technique relies on the microbes and their enzymes to degrade the substrate in the bags, and the assumption is the disappearance of the substrate is the effect thereof (López, 2009). Limitations of the latter technique include difficulty to standardise, labour-intensive, low reproducibility, inaccuracy for soluble or small particulate feeds and the requirement of fistulated animals (Hristov *et al.*, 2019).

Various *in vitro* methods have been used including the *in vitro* gas production technique (Menke *et al.*, 1979; Raab *et al.*, 1983) to evaluate rumen CP degradability, by recording the ammonia-nitrogen and gas production when feeds are incubated in buffered rumen fluid. Alternative methods to determine protein degradation have been proposed and include the three-step *in situ / in vitro* procedure (Calsamiglia & Stern, 1995) or the *in vitro* procedure by Ross *et al.* (2008). These methods are, however, all based on the work of Tilly & Terry (1963). Gargallo *et al.* (2006), verified the use of the Ankom Daisy^{II} (AD^{II}) incubator for ruminal protein degradation determination. Throughout the years, studies have confirmed that the AD^{II} incubator can also be used to predict the DM digestibility of forages, grains, and mixed rations for ruminants (Goering & van Soest, 1970; Holden, 1999; Brons & Plaizier, 2005; Damiran *et al.*, 2008; Mabjeesh *et al.*, 2010).

Therefore, in the current study, to mimic the *in vivo* processes for estimated ruminal CP degradation, the *in vitro* method using the AD^{II} incubator (Ankom Technology Corporation Fairport, NY, USA) was used. The advantage of using an *in vitro* method compared to *in vivo* is that it allows a simple, rapid estimation of the rate of degradation of the feedstuff while reducing frequent animal

handling (Ørskov *et al.*, 1980). Using the AD^{II} also improved labour efficiency and reductions in cost because large numbers of protein samples can be simultaneously assayed.

The objective of this study was thus to evaluate the different protein fractions and degradation of DDGS against other protein sources in an *in vitro* study, and to determine if DDGS is a viable option to replace other commonly used protein sources.

3.3. MATERIALS AND METHODS

3.3.1. General

Samples of five different protein sources, SBM, cottonseed meal (CSM), dried brewers grain (DBG), canola meal (CM) and DDGS were collected throughout South Africa, apart from the DDGS (maize origin) which came from Europe. The selection of the five samples was made because it is commonly used in the South African feed industry except for DDGS.

3.3.2. Sample preparation

Prior to *in vitro* incubation, representative samples (SBM, CSM, DBG, CM, DDGS) were milled with a standard laboratory mill (Scientec RSA Hammer mill Ser. Nr 372; Centrotec) through a 1.5 mm screen. According to Broderick & Cochran (2000), intermediate screen apertures (1.5–3 mm) are best suited for the *in sacco (*dacron bag) techniques.

Before the milled feed samples were used, they were sieved with a vertical shaker for five minutes to pass through a 106 µm sieve. The particles that were > 106 µm were subsequently used for analysis. The reason for this is, small particles (<100 µm) of ground feed samples may escape from the dacron bags (Bar Diamond, Parma, ID, USA) without being degraded and may lead to an overestimation of the zero-hour degradation values (Nel, 2012). According to Cruywagen (2007; unpublished data) after doing an image analysis on the dacron material of the bags, it was found that the pore sizes ranged from 31 to 99 µm, with a mean of 63 µm. Whereas Bar Diamond (Parma, ID, USA) claims the designated mean pore size is 53 µm ±10 standard deviation (SD). Huntington & Givens (1995) stated that a bag pore size smaller than 15 µm can decrease degradation because of lower microbial colonisation and diversity and trapping fermentation gases. Bag pore size larger than 40 µm can, however, cause losses of solubles and undegradable particles. Dacron bags of 200 mm x 100 mm were dried overnight at 55°C and placed in a desiccator for 30 min before weighing the dried bags and recording weights. Five (air dry) samples (± 8 g) were weighed in triplicate into dacron bags at the incubation times 0, 2, 4, 8, 16, 24, and 48 h (NRC, 2001). Coloured cable ties were used to close the double-folded open ends of the oven-dried bags (Cruywagen, 2006).

3.3.3. **Chemical analyses**

All the samples (SBM, CSM, DBG, CM, DDGS) were analysed according to the methods described by the Association of Official Analytical Chemists (AOAC, 2002) (Table 3.2):

- Moisture: Loss of drying (moisture) at 95 – 100 °C for feeds. (AOAC Official Method 934.01).
- Ash: AOAC Official Method 942.05.
- Crude fat: AOAC Official Method 920.39.
- CP: with the aid of the Leco protein analyser (LECO FP-528). (Crude protein in meat and meat products including pet foods. Combustion method. AOAC Official Method 990.03)
- Neutral detergent fibre (NDF) according to the method of Goering & van Soest, (1970) using the ANKOM 200/220 Fiber Analyzer.

The rumen digestibility of CP was calculated as the amount of CP extracted from the bag divided by the amount of CP in the sample.

3.3.4. **Preparation of the buffer solution**

The reduced buffer solution that was used in the *in vitro* method using the AD^{II} incubator was based upon the *in vitro* rumen degradability buffer solution described by (Goering & Van Soest, 1970). The composition of the van Soest Buffer used is presented in Table 3.1.

Table 3. 1 Components of Goering and van Soest (1970) buffer and reducing solution.

Buffer			
Reagents		**In H_20**	**/ litre * final**
De-ionised water		*(g/L)*	500 ml
Buffer Solution	NH_4HC0_3	4	250 ml
	$NaHC0_3$	35	
		(g/L)	
Macro-Mineral Solution	Na_2HP0_4	5.7	250 ml
	KH_2PO_4	6.2	
	$MgS0_4.7H_20$	0.6	
		(g / 100 ml)	
Micro-mineral solution	$CaCl_2.2H_20$	13.2	125 µl
	$MnCL_2.4H_20$	10	
	$CoCl_2.6H_20$	1	
	$FeCl_3.6H_20$	8	
Resazarin			1.25 ml
Tryptone			2.5 g
Reducing Solution			
Cysteine			0.3125 g
Potassium Hydroxide (KOH)			~ 12.5
Sodium Sulphide (NaS)			0.3125 g
Deionised water			50 ml

The buffer and reducing agent were purged with carbon dioxide (CO_2), sealed, and left in an incubator room at a temperature of 39˚C overnight. This process aided in preventing temperature shock to the rumen bacteria when the rumen fluid was added. On the day of inoculation, the reducing agent and buffer were combined before rumen fluid was collected. The reduction of the buffer could be observed by a change of colour from a pink or purple (oxidised) to a colourless solution (reduced) (Goering & van Soest, 1970).

3.3.5. **Animals and rumen fluid collection**

Rumen fluid inoculum was collected from three rumenally cannulated non-lactating Holstein cows at the facilities of the Welgevallen experimental farm of Stellenbosch University, in the Western Cape, South Africa. All rumen collections were done according to the rumen extraction protocol of the University of Stellenbosch and were approved by the Stellenbosch University's Animal Ethics Committee (reference: ACU-2020-10444). The cows received a commercial concentrate (12% CP) at a level of 2.5 kg twice daily (07:00 am and 03:30 pm) and grazed kikuyu (*Pennisetum clandestinum*) pasture. In the study done by Mabjeesh *et al.* (2010), the source of the inoculum did not affect the *in vitro* DM digestibility (*IV*DMD) of any of the feedstuffs used (which included protein sources like SBM, sunflower meal, corn gluten feed, CSM, CM, fish meal and whole cottonseed).

Rumen fluid, collected manually before afternoon feeding, was strained through a double layer of cheesecloth into three preheated (39.0 ± 0.5°C) thermal flasks filled to the brim to keep the contents anaerobic. The rumen fluid was immediately transferred to the laboratory and was strained for a second time through a clean double layer of cheesecloth into three separate preheated (39.0 ± 0.5°C) glass beakers. The beakers were purged with CO_2 as recommended by Goering & Van Soest (1970), sealed with rubber stoppers, and placed inside a preheated incubation chamber at 39.5°C. The pH was measured for each of the three cow's inoculum (pH of 6.7 ± 0.4 SD). In the experiments of Strobel & Russell (1986), they examined the effects of small changes in pH on rumen microbial protein synthesis. The latter authors reported that microbial protein synthesis was decreased ($P < 0.05$) at pH 6.0 and that even small declines in pH can be detrimental to rumen microbial protein synthesis. This may affect protein solubility (Hedqvist & Udén, 2006).

3.3.6. **The *in vitro* digestibility procedure**

Two separate runs were performed, where each protein sample had incubation times of 0, 2, 4, 8, 16, 24, and 48 h in three replicates of inoculum collected from three different cows resulting in six repetitions per feedstuff per time point. The experimental design was: 2 incubations × 5 feeds × 3 replications × 7 incubation times, giving a total of 42 values per feed, plus seven bags without a feed sample as blanks per replication to correct for rumen fluid. Three vessels per cow were used and filled with incubation inoculum, which consisted of diluted digesta inoculum with the buffer in a 1:4 (vol/vol) ratio as suggested in the original procedure of Goering and Van Soest (1970). Thus, 200 ml digesta inoculum and 800 ml buffer per vessel were used. Each of the vessels inside the AD^{II} contained a perforated septum that divided the content into two parts to allow the digestion medium to move freely inside (Tassone *et al.*, 2020). The weighed dacron bags containing feed samples (± 8 g/bag), including the blanks were placed in a specific digestion vessel. For this trial, each vessel (n=9) contained two of each of the protein feeds (the substrate for two time periods e.g., 2 h and 4 h; 8 h and 16 h; 24 h and 48 h) including the blanks, therefore 12 bags per vessel (see Figure 3.1).

The buffer solution (800 ml) and rumen inoculum (200 ml) were added to each vessel. The vessels were purged with CO_2, and the vessels (with lids) were placed into one of the two AD^{II} for incubation at 39°C with continuous rotation to facilitate the effective immersion of the bags for the duration of the specific incubation times. The bags were removed at the designated time intervals and then immediately washed with cold running tap water mixed with ice to stop the incubation process. For 0 h incubation, the dacron bags with feed samples were washed with cold water. After washing, the bags were dried in a forced-air oven at 55°C for 48 h, cooled in a desiccator for 30 min and were weighed. The content of each bag was removed and stored into plastic containers until analysis.

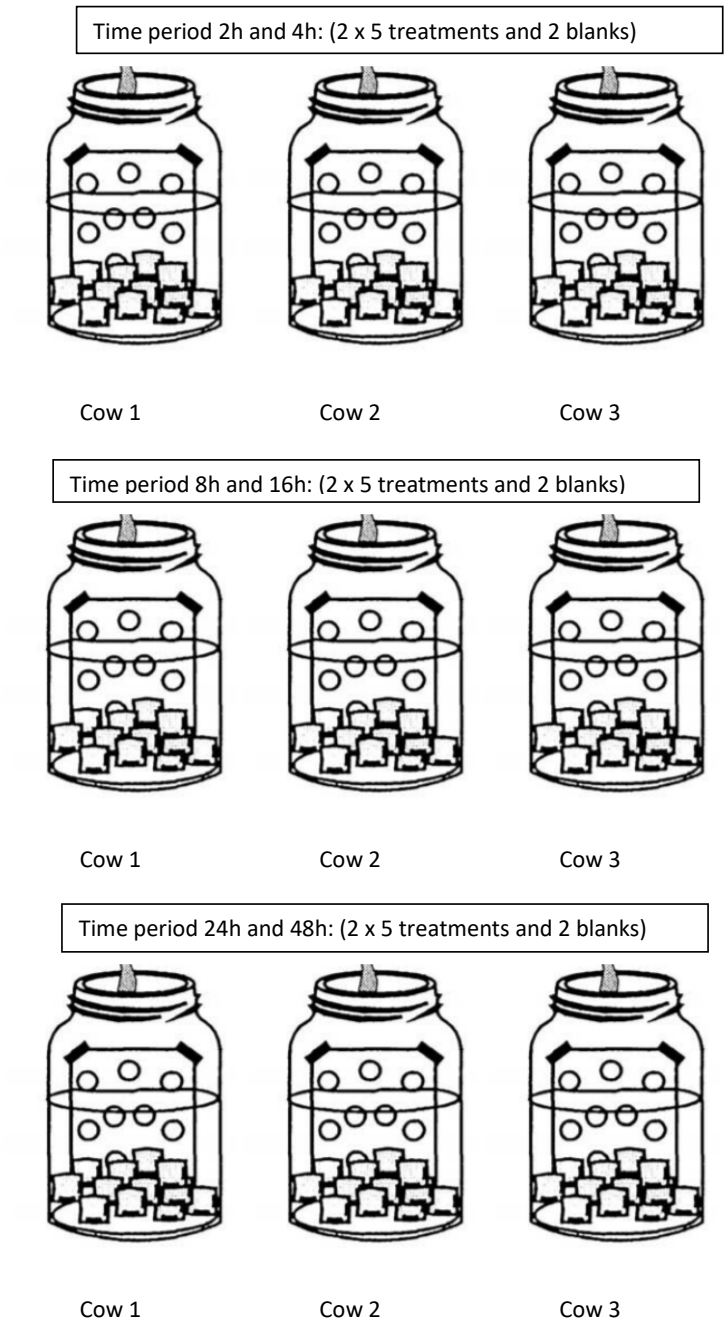


Figure 3.1 A visual description of the vessels with a perforated septum that divides the content into two time periods for when the dacron bags are removed.

3.3.7. **Calculations and statistical analysis**

An iterative least-square procedure was used (Solver function of Excel) to fit the CP disappearance data to the model of Ørskov & McDonald (1979) to determine degradability parameters without lag time:

$Y = a + b\ (1 - e^{-ct})$ **Equation 1**

Where Y = degradation at time t

a = soluble and rapidly degradable fraction

b = fraction that will potentially be degraded over time

c = rate of degradation of fraction b

The effective degradability (Ørskov & Mcdonald, 1979) of CP was determined as follows:

$D_{eff} = a + ((bc / (c + k))$ **Equation 2**

Where D_{eff} = Effective degradability; and

a, b, and c are the constants as described in equation 1; and

k is the rumen fractional outflow rate.

The effective degradability was calculated with an hourly disappearance coefficient of 0.05/h and 0.08/h. Data were recorded from t_0 to t_{48}. After 48 h of incubation, an asymptote was apparent, but not yet reached. To construct the respective charts to predict 72 h values, data were subjected to the trendline power chart option of Excel. For each raw material, all the x-values obtained from Solver results were used in the trendline power curve analyses and resultant regressions equations and R_2 values were used to predict y_{72}. Charts were then accordingly extrapolated.

Data were consequently analysed according to a main effects ANOVA with effects being treatment and block using STATISTICA 14, (2020). Treatment differences were considered significant at $P < 0.05$.

3.4. RESULTS AND DISCUSSION

3.4.1. Chemical composition of feed samples

As expected, the chemical composition of the five feedstuffs varied profusely and is presented in Table 3.2. The nutrient contents were, however, comparable to what the NRC (2007) reported. The two by-products, DDGS and DBG, were comparable in CP content (26.6% vs 29.4%), whereas the oilcake meals (SBM, CSM, and CM) contained more CP than DBG and DDGS. However, the fat percentage was higher in the distiller by-products, than in the oilcake meals. Dried distillers' grains with solubles had the highest fat content among the protein feeds (13.9%). Neutral detergent fibre values were the highest in DBG (64.7%) and the lowest in SBM (23.8%).

Table 3.2 Nutrient composition of the protein sources.

Parameter	DDGS (g/kg DM)	SBM (g/kg DM)	DBG (g/kg DM)	CSM (g/kg DM)	CM (g/kg DM)
DM	885	897	921	909	893
CP	266	483	294	413	352
Fat	139	15.6	78.8	43.2	32.5
Ash	45.0	63.9	30.8	67.7	65.9
NDF	442	238	647	365	325

DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, CM = canola meal, DDGS = distillers' grains with solubles.

3.4.2. Crude protein degradability of feed ingredients at different incubation times

As expected, the *in vitro* disappearance of CP from the bags incubated in the rumen fluid increased with increasing time (Figure 3.2 and Table 3.3). Among the protein feedstuffs, SBM, CM and DDGS proteins rapidly disappeared. Between 63% and 69% of their respective CP fractions disappeared during the first 16 h of incubation and illustrating a steep increase in the disappearance (Figure 3.2 and Table 3.3).

Table 3.3 The mean crude protein degradability of different protein feedstuffs after 0, 2, 4, 8, 16, 24, 48 h incubation in vitro (% DM).

Incubation	Treatment					SEM	*P* value
time (h)	DDGS	SBM	DBG	CSM	CM		
0	25.3^{a}	19.4^{b}	13.6^{c}	14.5^{bc}	21.2^{ab}	1.21	< 0.001
2	35.6^{a}	30.5^{a}	18.7^{b}	22.0^{b}	31.1^{a}	1.16	< 0.001
4	43.5^{a}	39.7^{a}	23.2^{b}	28.0^{b}	39.2^{a}	1.39	< 0.001
8	53.9^{a}	53.3^{a}	30.5^{c}	36.7^{b}	51.0^{a}	1.63	< 0.001
16	63.3^{b}	68.7^{a}	40.2^{c}	45.8^{c}	63.7^{ab}	1.67	< 0.001
24	66.3^{c}	75.7^{a}	45.8^{d}	49.6^{d}	69.1^{b}	1.76	< 0.001
48	67.7^{c}	80.9^{a}	51.9^{d}	52.0^{d}	72.8^{b}	2.21	< 0.001

SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, CM = canola meal, DDGS = distillers' grains with solubles. Means within the same row with different superscripts (a, b, c, and d) differ significantly ($P < 0.05$).

The CP disappearance values of CSM and DBG were significantly ($P < 0.001$) lower during the first 16 h of incubation approximately 46% and 40%, respectively. The CP disappearance after 24 h of incubation followed the same trend, with lower ($P < 0.001$) rates of disappearance resulting from CSM and DBG and higher ($P < 0.001$) rates of disappearance resulting from SBM, CM and DDGS. After 48 h the degradation of CSM and DBG were similar with no significant differences between each other, however, both were significantly ($P < 0.001$) lower compared with DDGS, SBM and CM (Table 3.3).

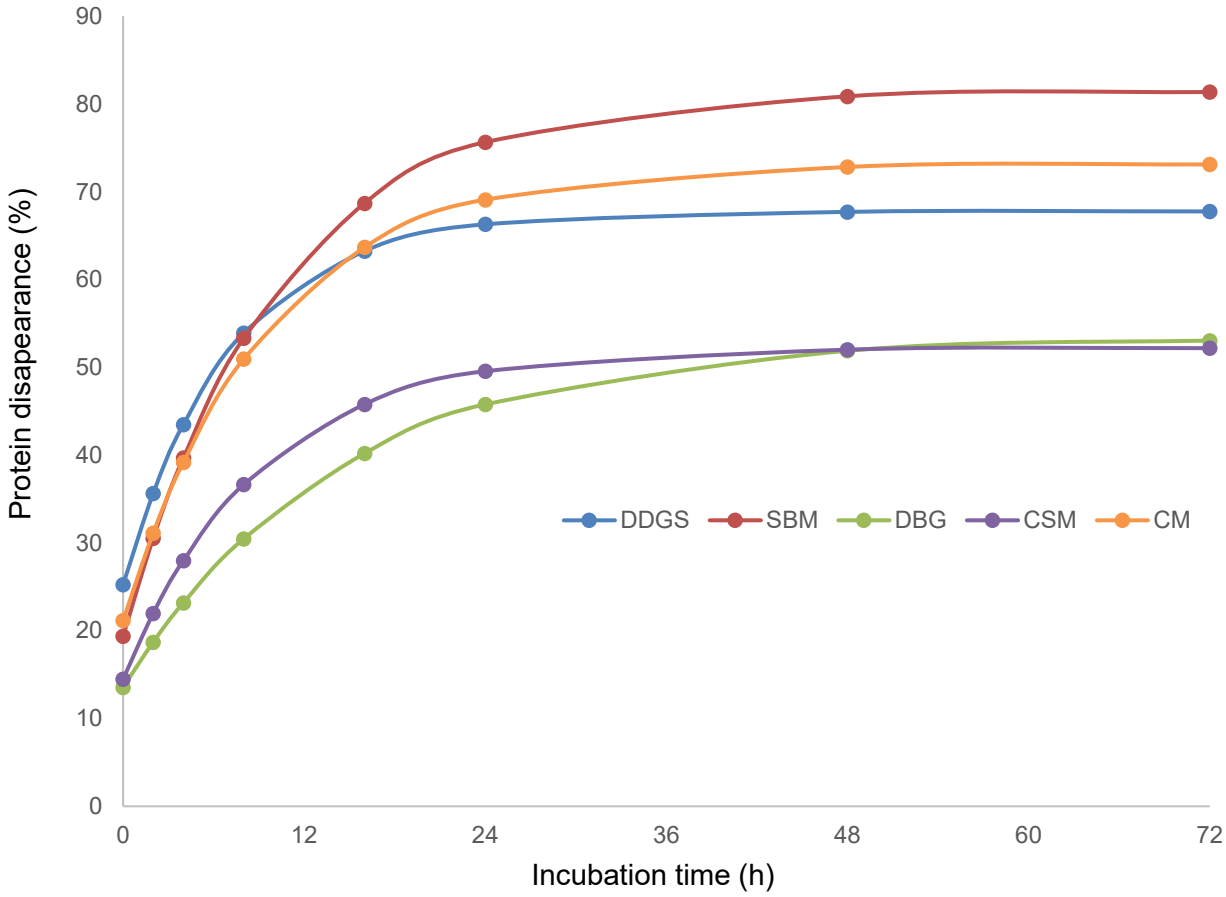


Figure 3.2 Predicted values of *in vitro* crude protein degradation for soybean oilcake meal (SBM), cottonseed oilcake meal (CSM), dried brewers grain (DBG), canola oilcake meal (CM) and dried distillers grains with solubles (DDGS).

3.4.3. **Ruminal degradation**

The mean values for effective degradability of CP at two different outflow rates are presented in Table 3.4. Effective degradability is defined as the degradability of a feed whilst considering the rate at which feed flows from the rumen to the small intestine (Woods *et al.,* 2003). The Agricultural and Food Research Council (AFRC, 1993) recommends: “an hourly disappearance rate of 2% per hour for animals with energy consumption lower than maintenance; 5% per hour for calves, cows, producing less than 15 kg of milk/day, and beef cattle and sheep with energy consumption less than twice of maintenance; and 8% per hour for dairy cows, producing over 15 kg of milk/day or with energy consumption more than twice the maintenance”. Madsen & Hvelplund (1985) preferred a passage rate (k_p) of 8%/h because it gave the closest relation to *in vivo* degradation.

The soluble fraction (a) of CP was greatest ($P < 0.05$) for DDGS (25.3%) and intermediate for CM and SBM (Table 3.4). No significant differences among CSM and DDGS and CM for this fraction were observed. However, significant differences between DDGS and SBM for fraction (a)

were established (Table 3.4). Small particle loss may have also contributed to the disappearance of CP from the bags, partially due to sample preparation, e.g., the milling of the sample (Maxin *et al*., 2013). The reason for the higher fraction (a) of the DDGS could perhaps be due to free amino acids produced from protein hydrolysis during the malting process. The rapidly degradable fraction (a) of SBM (19.4%) and DDGS (25.3%) found in the current study were higher than those reported by Lei *et al.* (2018), which were 11.8% and 13.8%, respectively. The differences between studies could be explained by Lei *et al.* (2018), using fistulated goats compared with cows in the current study.

Of the five evaluated feeds the lowest concentrations of soluble CP (fraction a) were observed with DBG (13.6%) and CSM (14.5%) and, although not significantly different from each other, they did differ significantly ($P < 0.05$) from the remaining protein feedstuffs. The NDF analysis of DBG has been the highest ($P < 0.05$) of the five feeds (Table 3.2), which could have influenced the *in vitro* fraction-a degradability. The CSM sample also contained reasonable amounts of lint in the sample. Significant variation ($P < 0.05$) between the feeds for the rate of degradation of fraction b, were observed with SBM differing from DDGS, DBG, CSM and CM. The highest and lowest degradable fraction (b) of CP was recorded for SBM (62.0%) and CSM (37.7%), respectively. No significant variation was found for the rate of degradation of the undegradable CP (fraction c) (Table 3.4). The CP degradation kinetics (a, b and c) and effective protein degradability's for CSM, SBM and CM obtained in this experiment were considerably lower than those obtained by Woods *et al.* (2003) and Hvelplund & Madsen, (1993). Differences could be attributed to the different chemical compositions and the manufacturing processes. According to Woods *et al.* (2003), a range of chemical components of the feeds were highly correlated to the degradability parameters (Ørskov & Mcdonald, 1979).

Table 3.4 The mean degradation parameters of crude protein in soybean meal, cottonseed meal, dried brewers grain, canola meal and distillers' grains with solubles.

Parameter	Treatment					SEM	*P*
	DDGS	SBM	DBG	CSM	CM		
a	25.3^{a}	19.4^{b}	13.6^{c}	14.5^{c}	21.2^{ab}	1.049	< 0.001
b	42.5^{c}	62.0^{a}	39.8^{c}	37.7^{c}	52.0^{b}	1.949	< 0.001
c	0.14	0.10	0.07	0.11	0.11	0.017	0.093
D_{eff} (0.05)	54.6^{b}	60.2^{a}	36.5^{c}	39.7^{c}	56.5^{ab}	1.094	< 0.001
D_{eff} (0.08)	50.3^{a}	53.3^{a}	31.9^{b}	35.7^{b}	50.8^{a}	1.025	< 0.001

SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, CM = canola meal, DDGS = distillers' grains with solubles. a: soluble protein fraction; b: slowly degradable protein fraction; c: undegradable protein fraction; Deff: effective degradability at two ruminal passage rates (0.05, and 0.08 h^{-1}). Means within the same row with different superscripts (a, b, and c) differ significantly.

Protein feed sources with high effective degradability of crude protein (EDCP) are those with high degradation levels in the rumen (Jafari Khorshidi *et al.,* 2013). The effective CP degradability values are in an inverse proportion to the passage rates from the rumen. The effective degradability of CP with k_p = 5% per hour was significantly higher ($P < 0.05$) in the oilcake meals, SBM and CM compared to the other protein meals (Table 3.4). Factors that might affect the degradability of protein could be the presence of fats and fibre in the feed. The higher fats in DDGS, DBG and CSM could possibly decrease the degradability of nitrogenous compounds (Doreau & Ferlay, 1995). The fibrous portion of the DBG and CSM reduces the degradability of feed (Busanello *et al.*, 2017). According to Liebe *et al.* (2018) models predicting RUP of feeds, NDF, CP, pore size, and the method of bagging the sample all had a significant effect on the measurement.

A similar trend was seen with the effective degradability of CP at k_p = 0.08 per hour, however, there were no significant differences ($P > 0.05$) between DDGS, SBM and CM. The RUP was calculated as % RUP = 100 - $\%D_{eff}$. The estimated RUP, assuming the passage rate of 0.08/h, ranged from 46.7% for SBM to 68.1% for DBG.

In a meta-analysis study, van Straalen (1995) compiled effective protein degradation of concentrate feedstuffs, determined by nylon bag incubations in different laboratories (Cronje, 1983; Shibui *et al.,* 1983; Boever *et al.,* 1984; Goetsch & Owens, 1985; Madsen & Hvelplund, 1985; Barrio *et al.,* 1986; Vérité *et al.,* 1987; Erasmus *et al.,* 1988; Tamminga & Ketelaar, 1988; Susmel *et al.,* 1989). Regression equations were calculated between the results in each data set with the same ingredients. The R squared regression equations ranged between 0.63 and 0.96. From these equations, corrected mean degradation values were calculated. The results of the current study (Table 3.4) were compared with the mean RUP of concentrate feedstuffs (obtained from the different laboratories, after correction for laboratory influence) of van Straalen (1995). The following comparisons and differences were observed: CSM (17 percentage units) and CM (10 percentage units) were higher in the current study compared to mean RUP values of 43% and 34%, respectively, (van Straalen, 1995) assuming the passage rate of 5%. The RUP values of SBM and DBG were closer to the van Straalen (1995) mean RUP values of up to four percentage units (39.8% and 63.5% respectively). The lowest EDCP values observed in the current study for DBG were expected, because it had both low protein solubility (fraction a) and degradability (Wohlt *et al.*, 1973; Armentano *et al.*, 1986).

Van Straalen (1995) reported that, although differences between laboratories were extensive, the sequence of degradation of the feedstuffs, however, were usually very similar (CM>SBM>CSM>DBG). In the present study, CM for both 5% and 8%/h rates were degraded to a lesser extent than SBM (Table 3.4). The higher EDCP for SBM was due a high insoluble but degradable fraction (fraction b). The reason why the values for degradation parameters of SBM were higher than the other feedstuffs could be due to relative high protein disappearance from the dacron bags. It is known as secondary particulate loss, where the new small particles that are formed (e.g.

<50 µm) during the break down of substrate may be lost during the incubation or rinsing (Huhtanen & Sveinbjörnsson, 2006). According to Can *et al.* (2011), oilseed processing frequently increases the proportion of digestible components in meals and produces protein supplements for animals of lower feed value. The extensive ruminal degradation of SBM (74% for whole soybeans and 71% for SBM) according to the NRC (2001), limits the utilization of soybean products by ruminants as sources of RUP.

The *in situ* EDCP fraction for maize-DDGS from European ethanol plants in the study of Westreicher-Kristen *et al.* (2012) with a rate of passage (k_p) of 8% per hour, was on average 61.3%, which is higher than the *in vitro* values from the current study (50.3%). Böttger & Südekum (2017) using *in vitro* methodology, compared various European DDGS samples. The latter authors reported RUP values of 33.4% and 40.7% at ruminal k_p values of 0.05/h and 0.08/h respectively. In the current study, the RUP values of 45.5% and 49.7% were obtained for k_p = 0.05 and 0.08, respectively. The chemical composition of DDGS can, however, vary significantly between manufacturers and across the many products available (Caldas *et al.*, 2020). A factor that may contribute to the variation is the differences in the heating process when producing DDGS since some parts of dietary proteins could be incorporated into fibre fractions by the amino-carbonyl or Maillard reaction (Kajikawa *et al.,* 2012). Other factors that could influence variability during ethanol production include grinding, cooking, fermentation, distillation, centrifugation, evaporation, blending and drying between production plants (Böttger & Südekum, 2018). As no information about the technical process was available in the current study, it does make it difficult to interpret the data. This could also be problematic for the feed industry to use DDGS effectively. The *in vitro* degradability from the current study (Table 3.4) of DBG and CSM were lower ($P < 0.05$) at both k_p = 0.05/h (38.1% ± 2.26 SE) and k_p = 0.08/h (33.8% ± 2.69 SE) compared with DDGS (54.6% and 50.3%, respectively), SBM (60.2% and 53.3%, respectively) and CM (56.5% and 50.8%, respectively).

3.5. CONCLUSION

In ruminant nutrition, protein supplements are expensive and represent an important portion of the diet. It is, therefore, imperative to find alternative protein sources of similar nutritional quality to conventional protein sources like SBM. It is furthermore important to determine the ruminal degradation of different feeds to specify the quality of the feeds. A feed ingredient with a low rumen degradability could increase the rumen bypass nutrients destined for small intestinal digestion with the aid of pancreatic enzymes in a similar fashion as in monogastric species. A limitation of using the dacron bag technique is that some protein fraction losses might occur due to small particle size losses during washing to determine 0 h values. This might result in an overestimation of RDP.

This study indicates that the observed RUP content (% of CP) at k_p = 0.08 was for SBM (46.7%) < CM (49.2%) < DDGS (49.7%) < CSM (64.3%) < DBG (68.1%). This sequence is comparable to data in literature except for SBM which was more degradable in the rumen than CM. Data from this *in vitro* trial, therefore, confirm that the ruminal degradation of DDGS and SBM were similar. Information collected from this study can be used by the industry as basic data for more accurate diet formulation for ruminants. This study confirmed that DDGS is a good dietary protein alternative for ruminants. However, given the high variability of dried distillers' by-products in South Africa, more studies should be done to compare the rate and extent of protein degradation with conventional protein sources.

3.6. REFERENCES

AOAC International. 2002. Official Method of Analysis. 17th ed. Association of Official Analytical Chemists Inc., Arlington, Virginia, USA.

Armentano, L. E., Herrington, T. A., Polan, C. E., Moe, A. J., Herbein, J. H., & Umstadt, P. 1986. Ruminal Degradation of Dried Brewers Grains, Wet Brewers Grains, and Soybean Meal. J. Dairy Sci. 69, 2124–2133. https://doi.org/10.3168/jds.S0022-0302(86)80644-0.

Barrio, J. R., Goetsch, A. L., & Owens, F. N. 1986. Effect of Dietary Concentrate on *In situ* Dry Matter and Nitrogen Disappearance of a Variety of Feedstuffs. J. Dairy Sci. 69, 420–430. https://doi.org/10.3168/jds.S0022-0302(86)80420-9.

Boever, J. L., Aerts, J. V, Cottyn, B. G., Vanacker, J. M., & Buysse, F. X. 1984. The *in sacco* protein degradability vs. protein solubility of concentrate ingredients1. Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkd. 52, 227–234. https://doi.org/10.1111/j.1439-0396.1984.tb00832.x.

Böttger, C., & Südekum, K. H. 2017. European distillers dried grains with solubles (DDGS): Chemical composition and *in vitro* evaluation of feeding value for ruminants. Anim. Feed Sci. Technol. 224, 66–77. https://doi.org/10.1016/j.anifeedsci.2016.12.012.

Böttger, C., & Südekum, K. H. 2018. Review: protein value of distillers dried grains with solubles (DDGS) in animal nutrition as affected by the ethanol production process. Anim. Feed Sci. Technol. 244, 11–17. https://doi.org/10.1016/j.anifeedsci.2018.07.018.

Broderick, G. A., & Cochran, R. C. 2000. *In vitro* and *in situ* methods for estimating digestibility with reference to protein degradability. Pages 53–85 in Feeding Systems and Feed Evaluation models. Theodorou, M.K., France, J., eds. CAB International, Wallingford, UK.

Brons, E., & Plaizier, J. C. 2005. Comparisons of methods for *in vitro* dry matter digestibility of ruminant feeds. Can. J. Anim. Sci. 85, 243–245. https://doi.org/10.4141/A04-072.

Busanello, M., Velho, J. P., Alessio, D. R. M., Haygert-Velho, I. M. P., Tambara, A. A. C., & Thaler Neto, A. 2017. In situ ruminal degradability of protein feeds with distinct physical forms: A meta-analysis. South African J. Anim. Sci. 47, 91–95. https://doi.org/10.4314/sajas.v47i1.13.

Caldas, J. V, Hilton, K., Mullenix, G., Xuemei, D., England, J. A., & Coon, C. N. 2020. Corn distillers dried grains with solubles : nutrient analysis, metabolizable energy, and amino acid digestibility in broilers. J. Appl. Poult. Res. 29, 1068–1083. https://doi.org/10.1016/j.japr.2020.09.015.

Calsamiglia, S., & Stern, M. D. 1995. ruminants A three-step *in vitro* procedure for estimating intestinal digestion of protein. J. Anim. Sci. 73, 1459–1465. doi: 10.2527/1995.7351459x.

Can, A., Hummel, J., Denek, N., & Südekum, K. H. 2011. Effects of non-enzymatic browning reaction intensity on *in vitro* ruminal protein degradation and intestinal protein digestion of soybean and cottonseed meals. Anim. Feed Sci. Technol. 163, 255–259. https://doi.org/10.1016/j.anifeedsci.2010.11.003.

Cömert Acar, M. 2018. Determination of ruminal protein degradation of three forages using *in vitro* protein fractions and *in situ* protein degradability characteristics. J. Dairy Vet. Anim. Res. 7, 154–159. https://doi.org/10.15406/jdvar.2018.07.00209.

Cronje, P. B. 1983. Protein degradability of several South African feedstuffs by the artificial fibre bag technique. S. Afr. J. Anim. Sci. 13, 225–228. Available at: https://www.ajol.info/index.php/sajas/article/view/139367.

Cruywagen, C. W. 2006. Technical note: A method to facilitate retrieval of polyester bags used in *in sacco* trials in ruminants. J. Dairy Sci. 89, 1028–1030. doi.org/10.3168/jds.S0022-0302(06)72169-5.

DAFF. 2020. Trends in the Agricultural sector 2019. Pretoria.

Damiran, D., DelCurto, T., Bohnert, D. W., & Findholt, S. L. 2008. Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets. Anim. Feed Sci. Technol. 141, 15–35. DOI:10.1016/j.anifeedsci.2007.04.007.

Das, L., Kundu, S., Datt, C., Kumar, D., & Datt, C. 2014. The evaluation of metabolizable protein content of some indigenous feedstuffs used in ruminant nutrition. Veterinary World. 7, 257-261

Das, L., Kundu, S., Datt, C., Kumar, D., & Tariq, H. 2015. *In situ* Ruminal Degradation Kinetics of Dry Matter, Crude Protein and Neutral Detergent Fiber of Tropical Ruminant Feedstuffs. Indian J. Anim. Nutr. 32, 45–51.

Doreau, M., & Ferlay, A. 1995. Effect of dietary lipids on nitrogen metabolism in the rumen: a review. Livest. Prod. Sci. 43, 97–110. https://doi.org/10.1016/0301-6226(95)00041-I.

Erasmus, L. J., Prinsloo, J., & Meissner, H. 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(1), 23-29. Available at: https://www.sasas.co.za/wp-content/uploads/2012/09/Erasmus18Issue1_0.pdf.

Gargallo, S., Calsamiglia, S., & Ferret, A. 2006. Technical note: A modified three-step *in vitro* procedure to determine intestinal digestion of proteins. J. Anim. Sci. 84, 2163–2167. https://doi.org/10.2527/jas.2004-704.

Goering, H. K., & Van Soest, P. J. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications) Agriculture Handbook No. 379. Agricultural Research Service, U.S.

Dept. of Agriculture., Washington, D.C.

Goetsch, A. L., & Owens, F. N. 1985. The effects of commercial processing method of cottonseed meal on site and extent of digestion in cattle. J. Anim. Sci. 60(3), 803-813. https://doi.org/10.2527/jas1985.603803x.

Hedqvist, H., & Udén, P. 2006. Measurement of soluble protein degradation in the rumen. Anim. Feed Sci. Technol. 126, 1–21. doi.org/10.1016/j.anifeedsci.2005.05.011.

Holden, L. . 1999. Comparison of methods of *in vitro* dry matter digestibility for ten feeds. J. Dairy Sci. 82(8), 1791–1794. https://doi.org/10.3168/jds.S0022-0302(99)75409-3.

Hristov, A. N., Bannink, A., Crompton, L. A., Huhtanen, P., Kreuzer, M., McGee, M., Nozière, P., Reynolds, C. K., Bayat, A. R., Yáñez-Ruiz, D. R., Dijkstra, J., Kebreab, E., Schwarm, A., Shingfield, K. J., & Yu, Z. 2019. Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. J. Dairy Sci. 102, 5811–5852. https://doi.org/10.3168/jds.2018-15829.

Huhtanen, P., & Sveinbjörnsson, J. 2006. Evaluation of methods for estimating starch digestibility and digestion kinetics in ruminants. Anim. Feed Sci. Technol. 130(1), 95–113. DOI:10.1016/j.anifeedsci.2006.01.021.

Huntington, J. A., & Givens, D. I. 1995. The *in situ* technique for studying rumen degradation of feeds. Nutr. Abstr. Rev. 65, 63–93. Available at: https://www.scirp.org/(S(351jmbntvnsjt1aadkozje))/reference/referencespapers.aspx?referenceid=1129634.

Huuskonen, A., Huhtanen, P., & Joki-Tokola, E. 2014. Evaluation of protein supplementation for growing cattle fed grass silage-based diets: a meta-analysis. Animal. 10, 1653–1662. https://doi.org/doi.org/10.1017/S1751731114001517.

Hvelplund, T., & Madsen, J. 1993. Protein systems for ruminants. Icel. Agr. Sci 7, 21–36.

Jafari Khorshidi, K., Abedi Chemazkoti, S., Kioumarsi, H., & Shariman Yahya, Z. 2013. Ruminal degradation of soybean, canola and cottonseed meal Uusing *in sacco* procedure in sheep. Pakistan J. Biol. Sci. 16, 898–900. DOI: 10.3923/pjbs.2013.898.900.

Kajikawa, H., Miyazawa, K., Yanase, A., Tanabe, Y., Tsuchida, Y., Mitsumoto, Y., Kozato, Y., & Mitsumori, M. 2012. Variation in chemical composition of corn dried distillers grains with solubles in relation to *in situ* protein degradation profiles in the rumen. Anim. Sci. J. 83, 299–304. https://doi.org/10.1111/j.1740-0929.2011.00956.x.

Lei, Y. G., Li, X. Y., Wang, Y. Y., Li, Z. Z., Chen, Y. L., & Yang, Y. X. 2018. Determination of ruminal dry matter and crude protein degradability and degradation kinetics of several concentrate feed

ingredients in cashmere goat. J. Appl. Anim. Res. 46, 134–140. https://doi.org/10.1080/09712119.2016.1276916.

Liebe, D. M., Firkins, J. L.,Tran, H., Kononoff, P. J., & White, R. R. Technical note: Methodological and feed factors affecting measurement of protein A, B, and C fractions, degradation rate, and intestinal digestibility of rumen-undegraded protein. J. Dairy Sci. 101, 8046–8053 https://doi.org/10.3168/jds.2018-14837

Liu, C., Li, D., Chen, W., Li, Y., Wu, H., Meng, Q., & Zhou, Z. 2019. Estimating ruminal crude protein degradation from beef cattle feedstuff. Sci. Rep. 9, 1–8. https://doi.org/10.1038/s41598-019-47768-3.

López, S. 2009. *In vitro* and *in situ* techniques for estimating digestibility. pp 87–121 in Quantitative aspects of ruminant digestion and metabolism. CABI, Wallingford.

Mabjeesh, S. J., Cohen, M., & Arieli, A. 2010. *In vitro* Methods for Measuring the Dry Matter Digestibility of Ruminant Feedstuffs: Comparison of Methods and Inoculum Source. J. Dairy Sci. 83, 2289–2294. https://doi.org/10.3168/jds.s0022-0302(00)75115-0.

Madsen, J., & Hvelplund, T. 1985. Protein degradation in the rumen. Acta Agric. Scand 25, 103–124. Availabe at: https://www.researchgate.net/publication/269406603_Protein_degradation_in_the_rumen/link/548ad33e0cf2d1800d7b2a76/download.

Maxin, G., Ouellet, D.R. & Lapierre, H., 2013. Ruminal degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distiller grains. J. Dairy Sci. 96 (8), 5151-5160

Menke, K., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. 93, 217–222. doi:10.1017/S0021859600086305.

Mohamed, R., & Chaudhry, A. S. 2017. Methods to study degradation of ruminant feeds. Nutr. Res. Rev. 21, 68–81. https://doi.org/10.1017/S0954422408960674.

Nel, M. 2012. The effect of fine particle removal on the estimation of protein degradability Parameters in dairy cattle MSc thesis, University of Stellenbosch, Stellenbosch, South Africa.

NRC. 2001. Nutrient Requirements of Dairy Cattle. seventh. The National Academies Press, Washington DC.

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

Ørskov, E. R., Hovell, F. D. D., & Mould, F. 1980. The use of nylon bag technique in the evaluation of feedstuffs. Trop. Anim. Prod. 5, 195–213. Available at: https://www.cipav.org.co/TAP/TAP/TAP53/53_1.pdf.

Ørskov, E. R., & Mcdonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92, 499–503. https://doi.org/10.1017/S0021859600063048.

Pacheco, D., Patton, R. A., Parys, C., & Lapierre, H. 2012. Ability of commercially available dairy ration programs to predict duodenal flows of protein and essential amino acids in dairy cows. J. Dairy Sci. 95, 937–963. https://doi.org/10.3168/jds.2011-4171.

Raab, L., Cafantaris, B., Jilg, T., & Menke, K. H. 1983. Rumen protein degradation and biosynthesis. I. A new method for determination of protein degradation in rumen fluid *in vitro*. Br. J. Nutr. 50, 569–582. doi: 10.1079/bjn19830128.

Ross, D. A., Gutierrez-Botero, M., & Van Amburgh, M. E. 2008. Development of an *in vitro* intestinal digestibility assay for ruminant feeds.in Cornell Nutrition Conference for Feed Manufacturers. pp 190-202. Cornell University, Ithaca, NY.

Ruzic-Muslic, D., Petrovic, M. P., Petrovic, M. M., Bijelic, Z., Caro-Petrovic, V., Maksimovic, N., & Mandic, V. 2014. Protein source in diets for ruminant nutrition. Biotechnol. Anim. HusbandryBiotehnologija u Stoc. 30, 175–184. https://doi.org/10.2298/bah1402175r.

Shibui, H., Kawai, T., Katoh, N., & Abe, M. 1983. Degradation properties of feed protein in the rumen of cows fed a high concentrate ration. Japanese J. Zootech. Sci. 511-517.

STATISTICA 14. 2020. TIBCO Software Inc. Data Science Workbench, version 14. http://tibco.com.

Van Straalen, W. M. 1995. Modelling of nitrogen flow and excretion in dairy cows. PhD thesis. University of Wageningen, Netherlands.

Strobel, H. J., & Russell, J. B. 1986. Effect of pH and Energy Spilling on Bacterial Protein Synthesis by Carbohydrate-Limited Cultures of Mixed Rumen Bacteria. J. Dairy Sci. 69, 2941–2947. https://doi.org/10.3168/jds.S0022-0302(86)80750-0.

Susmel, P., Stefanon, B., Mills, C. R., & Colitti, M. 1989. The evaluation of PDI concentrations in some ruminant feedstuffs: a comparison of *in situ* and *in vitro* protein degradability. Ann. Zootech. 38, 269–283. https://doi.org/10.1051/animres:19890307.

Tamminga, S., & Ketelaar, R. 1988. Eiwitbestendigheid van voedermiddelen voor herkauwers, The resistance against ruminal degradation of feeds for ruminants, report no. 192, IVVO, Lelystad, The Netherlands.

Tassone, S., Fortina, R., & Peiretti, P. G. 2020. *In vitro* techniques using the daisyII incubator for the

assessment of digestibility: A review. Animals 10, 1–24. https://doi.org/10.3390/ani10050775.

Tilly, J. M. A., & Terry, R. A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci. 18, 104-111. https://doi.org/doi.org/10.1111/j.1365-2494.1963.tb00335.x.

Vérité, R., Michalet-Doreau, B., Chapoutot, P., Peyraud, J. L., & Poncet, C. 1987. Revision du système des Protéines Digestibles dans l'Intestine (PDI). Bull. Tech. C.R.Z.V. 70, 19-34.

Westreicher-Kristen, E., Steingass, H., & Rodehutscord, M. 2012. Variations in chemical composition and *in vitro* and *in situ* ruminal degradation characteristics of dried distillers' grains with solubles from European ethanol plants. Arch. Anim. Nutr. 66, 458–472. https://doi.org/10.1080/1745039X.2012.740310.

Wohlt, J. E., Sniffen, C. J., & Hoover, W. H. 1973. Measurement of Protein Solubility in Common Feedstuffs. J. Dairy Sci. 56, 1052–1057. https://doi.org/10.3168/jds.S0022-0302(73)85304-4.

Woods, V. B., O'Mara, F. P., & Moloney, A. P. 2003. The nutritive value of concentrate feedstuffs for ruminant animals Part I: *In situ* ruminal degradability of dry matter and organic matter. Anim. Feed Sci. Technol. 110, 111–130. https://doi.org/10.1016/S0377-8401(03)00220-7.

Yildiz, E., & Todorov, N. 2014. The comparison of the main protein sources for dairy cows. A review. Bulg. J. Agric. Sci. 20, 428–446. Available at: http://agrojournal.org/20/02-28.pdf.

CHAPTER 4

THE EFFECT OF DIETARY PROTEIN SOURCES ON GROWTH PARAMETERS AND CARCASS CHARACTERISTICS OF FEEDLOT LAMBS.

4.1. ABSTRACT

The objective of this study was to compare dried distiller grains with solubles with commonly used protein sources, and evaluating growth, feed dry matter intake (DMI), feed conversion ratio (FCR), carcass characteristics and economical parameters of Dohne Merino lambs. The treatment diets were formulated to be iso-nitrogenous, iso-energetic and consisted of five different protein sources, namely soybean oil cake meal (SBM), dried distiller grains with solubles (DDGS), cottonseed oilcake meal (CSM), dried brewers grain (DBG) and a least-cost, optimally formulated diet (OPT). Thirty-five Dohne Merino wethers with an average initial weight of 28.5 ± 0,44 (SE) kg were allocated to a completely randomized design, with five treatments and seven replicates. At the end of the growth trial, the wether lambs were slaughtered, and their carcasses were evaluated. The final body weight and feed efficiency did not differ as a result of dietary treatment. Lambs did not differ in hot carcass weight and dressing percentage, although lambs fed soybean meal had 100% of carcasses with fat class 2 compared to the other treatment diets which had 86-71% of carcasses with a fat class 2 and the rest of the carcasses with a fat class 1. In the economic analysis, no significant differences were found among OPT and DBG, DDGS and SBM for the margin above specified cost. Dried distillers grains with solubles can therefore substitute commonly used protein sources used in feedlot lamb diets without negatively affecting feedlot performance and carcass quality or profitability.

4.2. INTRODUCTION

Protein sources in animal feed mostly originate from vegetable by-products (Chipa *et al.*, 2010). Soybean meal is the most popular protein source (DAFF, 2020) in the feed industry of South Africa, but because of the high cost (R8160/ton, SAPPO, 2021; Indexmundi, 2022), the need for researching alternative protein sources is required. The increase in soybean meal price in South Africa over the past 20 years is indicated in Figure 4.1

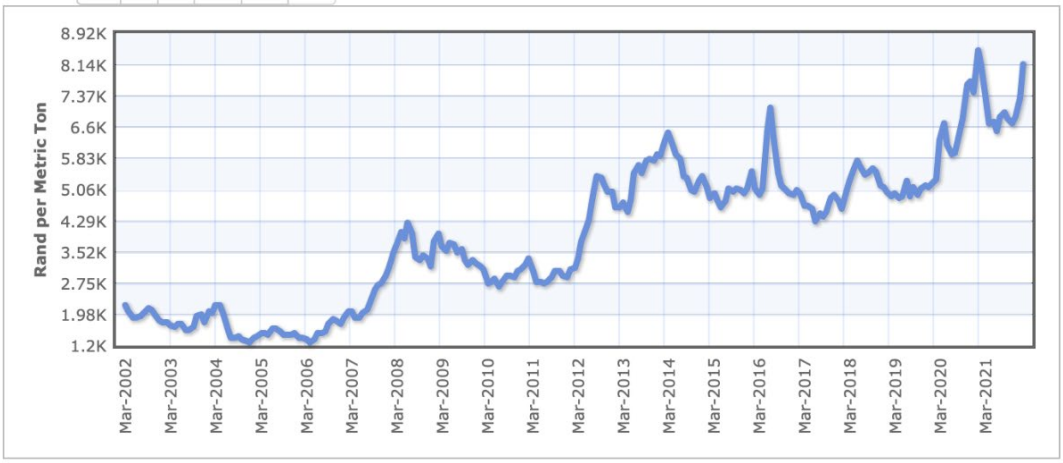


Figure 4.1 Soybean meal price (ZAR) in South Africa from March 2001 to March 2022 (Indexmundi, 2022).

The feed processing industry produces many by-products that could be used as alternative sources of protein for livestock. Depending on the different types of protein feeds, prices vary substantially (De Jager, 2016). Ignoring some tentative price variations, the average approximate cost of one metric ton in September 2021, was R 19400 for fishmeal (FM) with 65% CP, R 5 150 for canola oilcake (CM) with 35% CP and R 8200 for soybean meal (SBM) with 47% CP (SAPPO, 2021). According to Mabele Fuels (2021), dried distillers grains with solubles (DDGS) receives a price in the animal feeds market of between 0.80 and 1.2 times the price of maize. Compared to the protein feeds mentioned above, DDGS with 27% CP was therefore (in 2021) R 4128 per metric ton. Taking the protein content into consideration, the cost of a one-ton unit of crude protein (CP) was, therefore, R 12 610 (FM), R 1 803 (CM), R 3854 (SBM) and R 1 115 (DDGS).

This study aimed to compare dried distiller grains with solubles (DDGS) from the bio-fuel industry in terms of growth, dry matter feed intake (DMI), feed conversion ratio (FCR), carcass characteristics and economical aspects to other dietary protein sources in a lamb feedlot environment. This study was designed to determine if DDGS can replace SBM and other popular

protein sources used in a lamb feedlot. The hypothesis was that the production response of feedlot lambs would be similar when fed rations formulated with different protein sources, provided their protein, energy, vitamin, and mineral requirements are being met.

4.3. MATERIALS AND METHODS

4.3.1. **Location and facilities**

The trial was conducted at the Welgevallen Experimental Farm, managed by the Stellenbosch University, Faculty of AgriSciences, Stellenbosch. All procedures were approved by Stellenbosch University's Animal Care and Use Ethics Committee (Reference: ACU-2020-10444). The lambs were housed individually in a barn on slatted floors in 1.8 m x 1.2 m pens (Figure 4.2). Each pen was equipped with one feed trough and one water trough. All laboratory analyses were conducted at the Western Cape Department of Agriculture, Elsenburg. The trial was conducted from the end of September 2021 to the end of October 2021. Changing from winter to spring, fluctuations in temperatures occurred where the minimum temperature measured was 10ºC and the maximum was 21ºC.

Figure 4.2 The Welgevallen experimental farm feedlot facility.

4.3.2. **Animals and feeding management**

Newly weaned Dohne Merino wethers (n=35) with an average weight of 28.5 ± 0.44 (SE) kg and approximately 12 weeks of age, were sourced from the Overberg region in the Western Cape. Before arrival at the intensive feeding facility, they were administered an anthelmintic drench (Endotape®, Bayer Animal Health, Port Elizabeth, South Africa), vaccinated against *Clostridium Ovitoxicum* (Glanvac® 3, Zoetis South Africa, Sandton, South Africa), dosed with vitamins and minerals, (Oradose-A, SWAVET South Africa, Johannesburg, South Africa and Multimin® + Se + Cu Sheep and Angora Goats, Virbac South Africa, Centurion, South Africa) and received a visual ear tag. Each lamb was weighed before entering the different pens. The recorded weights were used for the allocation of animals to experimental treatments and to obtain a more homogeneous weight distribution amongst the different treatment groups.

The lambs had *ad libitum* access to oat chaff and fresh water. During the first two weeks, they were adapted to their respective concentrate feed (increased feed allocations with approximately 100 g per day). Apart from the feed adaptation, the first two weeks also help lambs to familiarise themselves with their surroundings and feeding routine (MLA, 2007). After the adaptation phase, the concentrate diets were provided *ad libitum* and fed twice daily at 07h00 and 16h00. The lambs had free access to fresh water throughout the study and no additional roughage was provided after adaptation. The wethers were observed twice daily for symptoms of discomfort, injuries and metabolic problems e.g., bloat or acidosis (diarrhoea, unthriftiness), or urinary calculi (frequent urination). Before feeding in the morning, the feed bunks were visually evaluated for feed leftovers of the previous day, the daily feed allotment was then adjusted (increased or decreased) in the afternoon feed to allow for a minimum of (<5%) feed accumulation. To calculate the feed conversion efficiency (FCE), the feed allocations and refusals were weighed and recorded weekly. The lambs were also weighed weekly. Individual average daily gain (ADG) was then calculated. The duration of the study was 45 days of which 14 days were used to adapt the animals to the treatment diets and pen environment and 31 days for data collection.

4.3.3. **Experimental design and dietary treatments**

The experiment was conducted using a randomized design Thirty-five lambs were dived into five (n=7) treatment groups. The experimental diets were randomly allocated to individual pens. With the pen being the experimental unit, animals housed individually ensured that the replicates were independent of each other (Seo *et al.*, 2018). Dietary treatments were formulated to meet the nutrient requirements of the animals according to NRC (2007) recommendations (Van Soest *et al.*, 1991; NRC 2007). Treatment diets were in a pelleted form and formulated to be iso-nitrogenous and iso-energetic (Table 4.1). The treatments consisted of five different protein sources: soybean meal (SBM), dried distiller grains with solubles (DDGS), cottonseed oilcake meal (CSM) and dried brewers

grain (DBG). A least-cost application was used to formulate an optimally balanced diet (OPT), with the inclusion of DBG and DDGS as the only crude protein sources. The OPT was included as the fifth treatment. Samples of the treatment diets were collected during the trial and stored at 4°C until analysed using AOAC accepted standard procedures for assays and analytical techniques as described in Section 4.4 (Table 4.1).

4.3.4. **Slaughtering procedures**

All the lambs were slaughtered at an average live weight of approximately 41 kg. At the end of the feeding period, the wethers were transported 80 km to be slaughtered at a commercial abattoir (Delico, Riebeeck-Kasteel). The animals were fasted over-night and slaughtered the next morning. The hot-carcass weights of the lambs were recorded on the day of slaughter, as well as the carcass classification and conformation. The lambs were weighed before slaughter. Because the feed and water were not withdrawn for 12h before weighing, and due to weight loss during transport to the abattoir, the initial and final weights were reduced (pencil shrink) by 4% to account for digestive tract fill (Cannas *et al.*, 2004). Standard South African slaughtering techniques were used as governed by the South African Meat Safety Act of 2000, which promotes electrical stunning at slaughtering to minimise pain and suffering (South African Government, 2000). The Carcass classification, according to age and backfat thickness (Government Notice R. 863 of 2006), was done by an independent, trained, and experienced carcass classifier approximately 20 minutes post-mortem and the individual hot carcass weights were measured. Cold carcass weights were recorded as a calculation of 4% less hot carcass weight (Muela *et al.*, 2010). According to Silva Sobrinho (2001), a mean value of 4% for cooling weight loss was found, based on data obtained from slaughterhouses and published papers.

4.4. CHEMICAL ANALYSES

Composite samples of representative samples of daily feed offered were collected throughout the feeding trial. It was ground through a 1 mm sieve with a laboratory hammer mill (Scientec RSA Hammer mill Ser. Nr 372; Centrotec). The samples were then analysed according to the methods described by the Association of Official Analytical Chemists (AOAC International, 2002) reported in Table 4.1.

- Analysis for DM, ash was determined by oven drying at 105°C (AOAC Official Method 934.01) and by incineration in a furnace at 550°C for 6 h, respectively (AOAC Official Method 942.05).
- The procedures for CP were done with the aid of the Leco nitrogen analyser (LECO FP-528) according to the crude protein in animal feed and pet food, AOAC Official Method 990.03.
- Ether extract (EE) with the AOAC Official Method 920.39.
- Neutral detergent fibre (NDF), acid detergent fibre (ADF) (Ankom220 Fiber Analyzer, ANKOM Technology, Fairport, NY, USA) according to the methods of Van Soest *et al.*, (1991).
- The nitrogen free extract (NFE) content was determined as 100 - (CP + EE + moisture + ash + CF).
- The starch content of the feed samples was determined according to the method described by Hall (2009).
- Total digestible nutrients (TDN) and Metabolic Energy (ME) content of feeds were predicted from their chemical composition values as per NRC (2001).

4.5. DATA MEASUREMENTS

4.5.1. **Feed consumption**

Fresh feed was weighed, recorded (“as fed” basis) and provided daily to the lambs. The refusals were weighed once a week and were recorded during the experimental period for each of the lambs. Feed samples were also taken throughout this period and were sub-sampled for chemical analysis. The feed intake of each lamb was calculated as a difference between the feed fed and refusals (DM basis) and was recorded weekly.

4.5.2. **Growth performance and feed efficiency**

Final body weight, weekly body weight change, average daily body weight gain (ADG) and the feed conversion ratio was recorded and calculated. The lambs were weighed individually before the commencement of the experimental period and then every 7 days.

4.5.3. **Average daily gain**

The weekly weight was used to calculate the average daily gain (ADG) per week of the lambs. The calculation of the ADG over the whole experimental period was as follow (Zhang *et al.*, 2021):

$$\mathrm{ADG} = \frac{(BW_{\mathrm{i}} - BW_{\mathrm{f}})}{N}$$

Where BW_i and BW_f are the initial body weight at the start of the experimental period whereas final body weight is the weight at the end of the experimental period, and N is the number of feeding days.

4.5.4. **Feed conversion ratio**

Feed efficiency can be measured by various methods, one of them is feed conversion ratio (FCR), which was calculated as the total dry matter intake (DMI) divided by the weight gain (ADG) (Lima *et al.*, 2017).

$$\mathrm{FCR} = \frac{DMI}{ADG}$$

4.5.5. **Carcass characteristics**

At the abattoir, individual hot carcass weight and calculated cold carcass weight, and classification were measured and recorded. Dressing percentages were calculated from cold carcass weight as a percentage of final live weights including fleece (Sheridan *et al.*, 2003). It is expressed as a percentage of the live weight of the animal according to the following formula as used by Warriss (2000).

$$\text{Dressing percentage } \% = \frac{Cold\ Carcass\ Weight}{Live\ Body\ Weight} \times 100$$

4.5.6. **Economic analyses**

Having the feed price per ton (DM), the total feed intake (DM), the cold carcass weights (kg), the classification of the animals as well as the carcass price per class on the day of slaughter (R/kg), the income and feed cost as well as the margin above specified costs (R/head), could be calculated for each lamb.

4.6. STATISTICAL ANALYSIS

Data pertaining to growth performance, carcass and economic performance were subjected to a one-way ANOVA using STATISTICA 14 (2020), with pen as the experimental unit. Data recorded over time (feed consumption, weight gain and feed efficiency) were analysed according to a repeated measures ANOVA. The Shapiro-Wilk test was used to check for normality and homogeneity of variance. The initial live weight was not included as a covariate in the model because the effect was not significant for average daily gain (ADG). Furthermore, according to a Levene's test, the variation in initial live weight amongst treatments did not differ significantly. The least significant difference (LSD) was calculated at the 5% level to compare treatment means. The resultant differences were deemed significant at $P < 0.05$.

4.7. RESULTS AND DISCUSSION

4.7.1. **Nutrient composition of feedstuffs**

The composition and analysis of the five treatment diets presented in Table 4.1 was formulated to be iso-nitrogenous and iso-energetic. Dry distillers grain with solubles have a slightly lower inclusion of maize compared to the other treatment diets because of higher fat content thereof (see Section 3.3.2). The advantage of this is that the removed starch reduces the potential to cause acidosis (Yildiz & Todorov, 2014). Sugar cane bagasse was included to obtain a desirable level of fibre in the diets. As expected, a lower level of sugar cane bagasse pith was included in Treatment 1 due to the NDF content of DBG being higher compared to the other protein sources (see section 3.3.2.). The high NDF content of DBG is because it is mainly composed of the husk pericarp and seed coat. These components are relatively high in lignin, cellulose, hemicellulose, lipids, and protein (Ikram *et al.*, 2017).

Table 4.1 Feed ingredients and chemical composition of the treatment diets containing DBG, DDGS, CSM, SBM and OPT.

	Treatment[3]				
	1	**2**	**3**	**4**	**5**
Ingredients[1] (% as fed basis)	**DBG**	**DDGS**	**CSM**	**SBM**	**OPT**
Maize	46.3	42	57.8	49.8	46.4
Brewers Grain	18.4	0	0	0	14.3
DDGS	0	23.8	0	0	4.8
Cotton Oilcake meal	0	0	10.8	0	0
Soya Oilcake meal	0	0	0	12	0
Sugar cane bagasse	14.7	18.7	18.3	19.7	16.4
Molasses	8.9	9	9	9	9
Wheat bran	7.6	2.3	0.04	6	5
Feed lime	1.3	1.7	1.3	1.3	1,3
Urea	1.0	0.64	1	0.35	1
Ammonium Chloride	0.8	0.8	0.8	0.8	0.8
Salt Fine	0.5	0.6	0.5	0.5	0.5
Mineral, Vitamin and Feed additive Premix[2]	0.5	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**	**100**

Nutrients (DM)	**1**	**2**	**3**	**4**	**5**
	DBG	**DDGS**	**CSM**	**SBM**	**OPT**
DM (g/kg)	898.2	883.2	902.4	902.0	892.7
Ash (g/kg)	60.1	85.5	63.5	65.6	102.6
CP (g/kg)	114.7	110.9	129.1	116.3	111.3
Crude fat (g/kg)	32.2	37.5	20.3	23.9	32.4
NDF (g/kg)	264.8	255.0	260.8	210.8	235.5
ADF (g/kg)	155.7	163.9	161.8	121.3	152.2
ME (MJ/kg)	10.74	10.36	10.55	10.88	10.27
NFE (g/kg)	566.7	533.0	567.9	612.1	544.5
Starch (g/kg)	415.1	411.5	408.1	438.6	433.8
TDN (g/kg)	716.7	690.8	704.1	725.9	685.4

[1]Ingredients: Ingredient composition of the five experimental diets, on an “As Is” basis.
[2]Composition per kg contained: Magnesium, 21 g; sulphur, 14 g; potassium, 434 g; manganese, 3585 mg; zinc, 5135 mg; iron, 1 mg; copper, 47 mg; cobalt, 27 mg; iodine, 48 mg; selenium, 6 mg; vitamin A, 777000 IU, Zinc Bacitracin, 3000 mg; salinomycin, 4200 mg. [3]Treatment: Specific protein concentrate included in the experimental diet. DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ME = metabolic energy; NFE = Nitrogen free extract; TDN = Total digestable nutrients; SBM = soybean meal, CSM = cottonseed meal, DBG=dried brewers grain, OPT= optimal diet, DDGS = distillers grains with solubles.

Sugar cane bagasse (40-43% CF) dry material consists of two major physically identifiable constituents, namely the hard sturdy fibres of the outer cortex and the inner pith or parenchyma which contained the original juice (Van Niekerk, 1981). Out of these two components, the pith is often preferred as it acts as the ideal carrier for molasses and it also has a better appearance in blended feeds (Van Niekerk, 1981). Nevertheless, according to (Kirk *et al.*, 1969) there was no difference in rate or efficiency of gain with beef cattle when 15% bagasse was compared with other roughage sources.

Despite minor differences in the chemical composition of the diets (Table 4.1) analysed they were relatively similar. Treatment 4 (SBM) was slightly lower in NDF content (21.08%) and ADF content (12.13%) while the overall average was 24.53% and 15.1% respectively. This corresponds to the NDF content of SBM, which has the lowest NDF content compared to the other protein sources used (Table 3.2). The CP concentration was higher for SBM and CSM, compared to DDGS, DBG and OPT, therefore variable amounts of non-grain feedstuffs (i.e., wheat bran and urea) were used to formulate the experimental diets iso-nitrogenous. The CP content (12.9%) of treatment 3 (CSM) was to some extent higher compared to the other treatment diets (overall average of 11.65%). Despite the slight variation in diet CP concentrations, the CP concentrations of all the treatment diets were lower than the diets expected formulated value of 14.5 % and what the NRC (2007) and NRC (1985) recommend for finishing early-weaned lambs fed for maximum growth. The NRC (1985) does not consider the rumen undegradable intake protein requirement, which is important to take into consideration for rapidly growing animals because of their high post-ruminal requisite for amino acids (Beauchemin *et al.*, 1995). Furthermore, lambs fed undegradable protein had a higher feed intake as well as the rate of gain compared to diets with diets lower in RUP (rumen undegradable protein) (Leng *et al.*, 1981; Hassan & Bryant, 1986).

Lambs can compensate for low levels of crude protein and/or ME (MJ ME /kg DM) in the diet to some degree, by increasing dry matter intake, but their capacity to do that will be limited by the rate at which the diet is degraded in the rumen and rumen outflow rate (MLA, 2010). However, Han *et al.* (1996) formulated concentrates taking into consideration the growth stages and length of the experiment. In the latter author's feed trail, two different concentrates were used; concentrates formulated for growing sheep were used for the first 4 weeks with approximately 15% CP (DM basis), and that for fattening for the remaining 4 weeks containing 11% CP (DM basis). The latter protein feed levels were similar to the current study.

With intensive feeding systems and the short time on feed, the lambs' finishing diets usually contain 35 to 45% of starch to increase diet energy density (Ferret *et al.*, 2008). Comparing the starch levels of the five treatment diets in this study, the SBM and OPT did have a reasonably higher level compared to the rest of the treatments (43.86% and 43.38% compared to the overall average of 42.34%). In the study of Oliveira *et al.*, (2017) they evaluated the effects of diet on growth and carcass amongst other parameters, of forty lambs when fed high (50%) and mid starch (35%) diets.

The latter authors concluded that the lambs fed high-starch diets had lower feed intake (DMI) (0.83 vs. 0.97 kg/d, P = 0.023) than those fed mid-starch diets. However, starch content had no impact (*P* > 0.05) on ADG (averaging 307 g/d), slaughter live weight (averaging 32 kg) or hot carcass weight (averaging 15.4 kg). Therefore, high-starch treatments improved the production performance and increased (*P* = 0.021) the gain to feed ratio by 0.05 points compared with mid-starch treatments.

4.7.2. **Feed consumption**

Feed intake is important as it directly influences the production potential and efficiency of the animal, if the animal consumes more feed, its daily production will increase, which in turn is beneficial due to the reduction of maintenance costs as productivity rises (McDonald *et al.*, 2002). The limitation of excessive intake of feed however includes high-fat accumulation, which should be avoided because of lower consumer interest and because it is expensive to produce (Forbes, 2007).

No signs of acidosis, bloat or urinary calculi were observed with any of the treatment diets. The mean daily dry matter intake (DMI) of the experimental lambs, measured weekly, is presented in Table 4.2 and Figure 4.3. The mean daily DMI (1513 g/lamb) was similar compared to the study of Van der Merwe *et al.* (2020a) who reported 1506 g/lamb for Dohne Merinos. The DMI of the current study however was higher compared to what was observed from a growth trail of SA Mutton Merino lambs conducted by Brand & Van der Merwe (1993) of 1147 g/lamb/d. The average daily DMI of lambs was 1527 g, 1409 g, 1504 g, 1510 g and 1616 g for DBG, DDGS, CSM, SBM and OPT, respectively (Table 4.2). This is slightly higher than what the NRC (1985) recommends (1400 g/d for 30kg lambs, growing 325 g/d). The DMI recorded in the current study was within range of the recommended levels of the NRC (2007), for growing lambs, which was between 1006 g - 1550 g DM/lamb. As presented in Table 4.2 and Figure 4.3, an upward trend in mean daily DMI can be seen over the 4-week of the experimental period. Significant differences (*P* < 0.05) in DMI were observed among treatments OPT and to DDGS during the feeding trial (Table 4.2). The slight decline in DMI during the third week of the feed trial corresponds to the increase in average daily temperatures (above 20ºC) for that period. Heat stress causes animals to reduce DMI to decrease increased heat production associated with fermentation of the feed, which also affects the performance of the animals (Morrison, 1983).

Table 4.2 The mean DMI over four weeks for the different treatment diets.

Item	Period[2]	Treatment[1]					
		DBG	DDGS	CSM	SBM	OPT	SEM
DMI (kg/d)	1	1.34^{ab}	1.17^{b}	1.35^{ab}	1.52^{a}	1.43^{a}	0.073
	2	1.47^{ab}	1.44^{b}	1.54^{ab}	1.44^{b}	1.66^{a}	0.064
	3	1.58^{a}	1.35^{b}	1.52^{ab}	1.50^{ab}	1.63^{a}	0.074
	4	1.66^{ab}	1.46^{b}	1.59^{ab}	1.58^{ab}	1.70^{a}	0.074
	mean	1.52^{ab}	1.40^{b}	1.52^{ab}	1.51^{ab}	1.62^{a}	0.053

[1]Treatment: Specific protein concentrate included in the experimental diet. SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, OPT= optimal diet DDGS = distillers' grains with solubles 2 Periods were 1 week in length. Means within a row without a common superscript letter differ ($P < 0.05$).

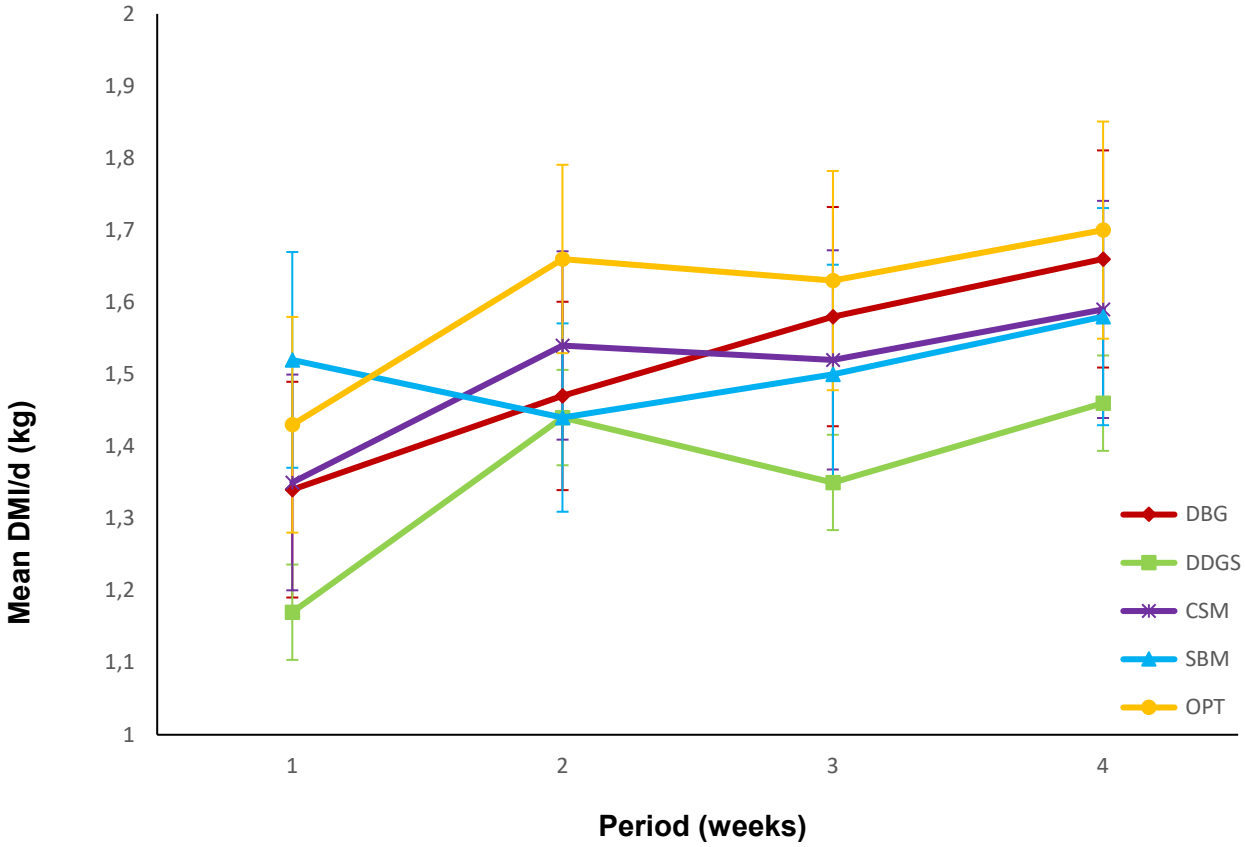


Figure 4.3 The Mean daily DMI of the lambs on different treatment diets over the experimental period of 4 weeks. Error bars represent the SE.

In previous studies where lambs received different levels of RUP in iso-caloric and iso-nitrogenous diets, the DMI increased linearly with increasing dietary RUP concentration (Haddad *et al.*, 2005; Akhtar *et al.*, 2016). In Chapter 3 the respective protein raw materials had the following estimated RUP concentrations, assuming k_p = 0.08/h: 46.7% for SBM; 49.7% for DDGS; 64.3% for

CSM and 68.1% for DBG. The lambs fed the treatment diet containing DDGS had the lowest mean DMI, compared to the other treatment groups. In goats, Gurung *et al.* (2009), suggest that when the concentration of fat surpass 7-9% of the diet, it may depress DMI, however in the current study the fat did not exceed that level. In the study done by Schauer *et al.* (2008), the authors similarly reported that supplemental fat from the DDGS may have affected intake and performance in their study. Data form Chapter 3 indicated that DDGS indeed contained a higher fat content than the other raw materials used in this study and the fat content was also marginally higher in the experimental diets containing DDGS (Table 3.3).

Furthermore, subjective visual appraisal of the different treatment diets, DDGS did indeed seem to have higher levels of pellet breakage and formation of fines compared with the other treatment diets. The reason for the higher amounts of pellet breakage in the DDGS treatment diet could have been because of a lower maize inclusion level combined with higher fat content. Starch plays an important function, amongst others, as an adhesive or binding agent, when subjected to heat treatment (gelatinizing of starch) (Smith, 1983). Additionally, this diet (T2) had the highest fat content and added to the starch suspension may also influence the pellet quality by reducing the volume of the produced gel (Eliasson, 1981). The high amounts of fines and breakage of the treatment diet containing DDGS may have decreased DMI. In an experiment conducted by Li *et al.* (2021), lambs consumed 34% more ($P < 0.001$), when pelleted feed was provided compared to un-pelleted feed (half of TMR was pelleted, and the other half was kept un-pelleted as loose mash). According to Li *et al.* (2021), pellets result in higher DMI due to the reduction in rumen fill, which allows greater feed intake to reach satiety. In addition, feeds that are dusty may irritate the nose and eyes of animals, and therefore decrease feed intake (Preston & Leng, 1987).

4.7.3. **Weight gain**

The results of body weight gain over the experimental period between different treatment diets are depicted in Table 4.3 and Figure 4.4. The bodyweight of all the lambs increased steadily to the end of the trial for all the treatment diets (Figure 4.4). Neither initial nor final BW differed significantly among treatments. The highest mean final body weight was achieved by lambs in the OPT treatment. There were no significant differences in final BW among lambs fed the diets containing DBG, CSM, SBM and OPT during the feed trial. However, the BW of lambs that received DDGS was significantly lower ($P < 0.05$) compared to the BW observed in lambs on the diets containing CSM, SBM and OPT in the third week of the feeding trial (Table 4.3). A sharp rise in weight between days 21 and 31 for the lambs fed DDGS (Figure 4.4) suggests that the prior week's degree of DMI reduction (Figure 4.3,) which may have been caused by pellet breakages and or higher temperatures, likely resulted in compensatory growth (Mahyuddin, 2009). Compensatory growth is defined as a physiological process whereby animals undergo a period of accelerated

growth following a period of restricted growth (Hornick *et al.*, 2000). According to (Jafarnejad *et al.*, 2010) a few factors affect optimal growth rates which include environmental temperature, diet nutrient density and physical feed quality.

Table 4.3 The mean body weight gain of lambs receiving different treatment diets.

Item Body weight (kg)	**Period**[2]	**Treatment**[1]					**SEM**
		DBG	**DDGS**	**CSM**	**SBM**	**OPT**	
Initial BW		30.7	30.3	32.7	31.9	31.7	0.981
	1	32.8	32.0	34.7	34.3	34.5	0.913
	2	35.8^{ab}	34.7^{b}	37.7^{a}	36.7^{ab}	37.2^{ab}	0.944
	3	37.6^{ab}	36.0^{b}	39.3^{a}	38.8^{a}	39.1^{a}	0.996
Final BW	4	39.7	39.6	41.6	41.1	42.2	1.047

[1]Treatment: Specific protein concentrate included in the experimental diet. SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, OPT= optimal diet DDGS = distillers' grains with solubles
[2]Periods were 1 week in length. Means within a row without a common superscript letter differ ($P < 0.05$).

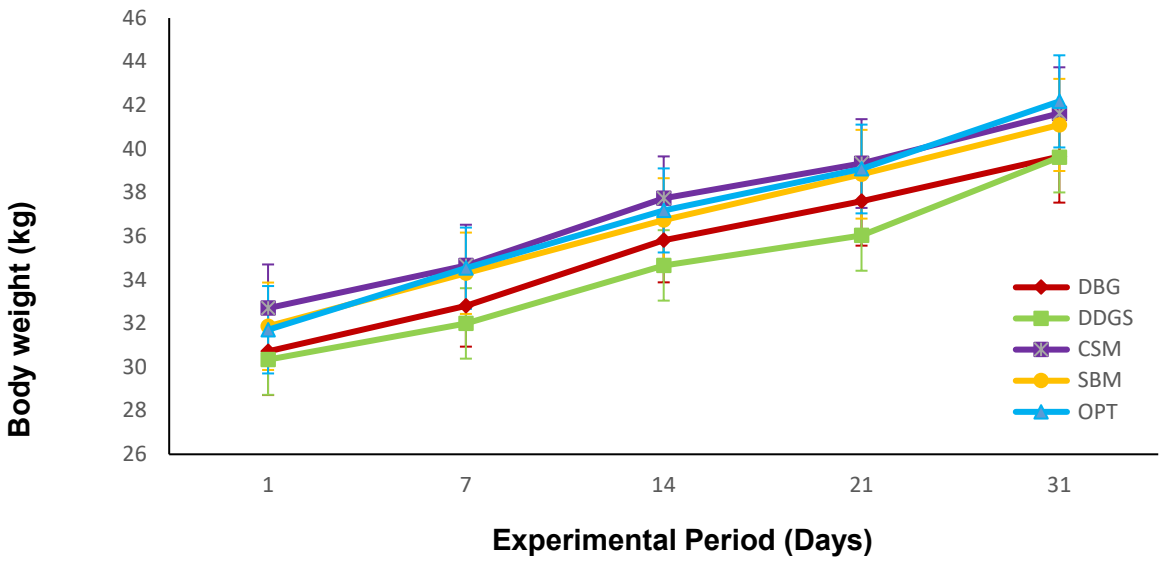


Figure 4.4 The mean body weight of the lambs on different treatment diets over the experimental period of four weeks. Error bars represent SEM.

4.7.4. **Growth performance and gain efficiency**

The overall ADG observed across treatments for the total experimental period in the current study was 302 g/day and was comparable to that established by the NRC (2007) for lambs in this category and did not differ significantly ($P > 0.05$) among treatments. The lack of significance in the current study could have been due to the limited number of animals (n=7). The lambs fed diets containing DDGS had an ADG of 299 g/d, which is slightly below the total group's overall mean, but it also had the lowest total feed intake of all the experimental diets, which positively affects the FCR. The results for the mean daily gains in this trial are slightly higher compared to the feedlot trial of Grimsell (2020) who reported a minimum mean ADG of 241 g/lamb for the canola meal treatment, and a maximum mean ADG of 287 g/lamb for the DBG treatment fed to Dohne Merino lambs. The NDF levels of the experimental diets reported by Grimsell (2020) were, however, higher than that of the present study. This could have contributed to lower DMI and therefore lower ADG values reported by Grimsell (2020). According to Ahmad *et al.* (2014), diets with high NDF levels may have an opposing effect on DMI of ruminants. The lambs used in the study of Grimsell (2020) were also older (22 weeks) compared to the lambs in the current study (12 weeks) and could have contributed to the difference in DMI observed between the two studies. In the study of Malik *et al.* (1996) the effect of age was significant for ADG in lambs. The latter authors concluded that there was a trend for ADG to increase from 9 to 21 weeks (214-237 g) and decrease from 21 to 29 weeks (237- 174 g). Table 4.4 and Figure 4.5 illustrate the fluctuation of ADG during the experimental period. During the first week of the feedlot trial, there was significant variation in ADG ($P < 0.05$) between diets containing DDGS (238 g/d) and OPT (402 g/d). This corresponds to the DMI intake levels of these treatment diets during the first week (Figure 4.3). However, during the last weeks of the feed trial, the ADG of lambs on treatments DDGS and OPT were significantly higher than the other three treatment diets ($P < 0.05$).

Table 4.4 The mean daily gain of the lambs on different treatment diets over the experimental period of four weeks.

Item		**Treatment[1]**					
	Period[2]	**DBG**	**DDGS**	**CSM**	**SBM**	**OPT**	**SEM**
Average daily gain (kg)	1	0.297^{ab}	0.238^{b}	0.279^{b}	0.347^{a}	0.402^{a}	0.033
	2	0.429	0.379	0.439	0.347	0.379	0.049
	3	0.256	0.197	0.229	0.301	0.270	0.038
	4	0.292^{c}	0.512^{a}	0.329^{bc}	0.320^{c}	0.443^{ab}	0.047

[1]Treatment: Specific protein concentrate included in the experimental diet. SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, OPT= optimal diet DDGS = distillers' grains with solubles. [2]Periods were 1 week in length. ^{abc} Means within a row without a common superscript letter differ ($P < 0.05$).

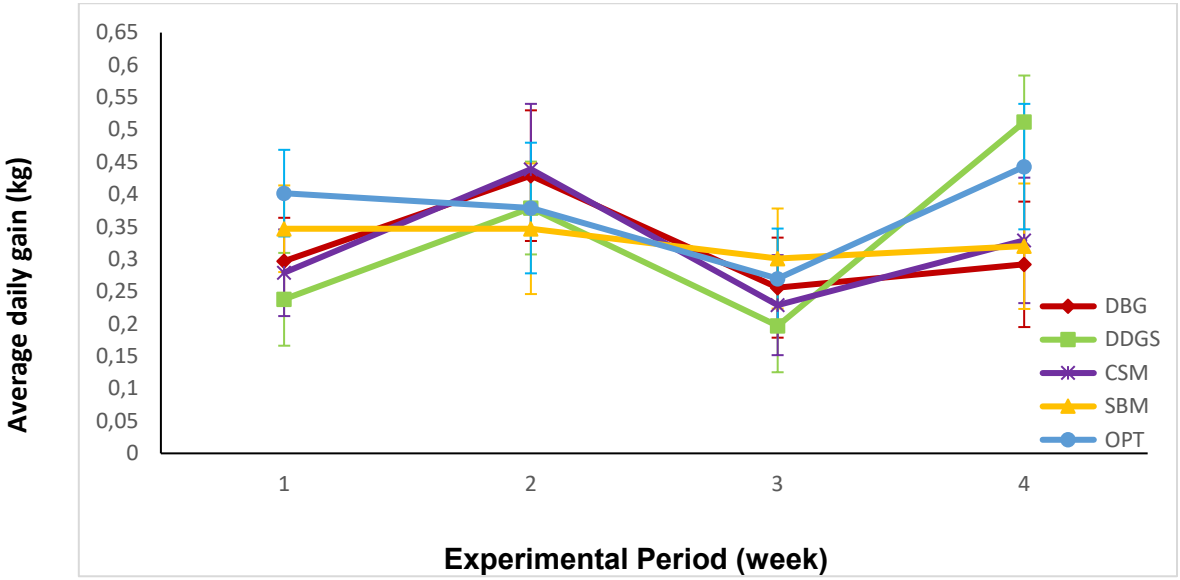


Figure 4.5 The average daily gain of the lambs on different treatment diets over the experimental period of 4 weeks. Error bars represent the SE.

In a thorough meta-analysis, Klopfenstein *et al.* (2008) suggested an inclusion level of 20% of DDGS for feedlot cattle to obtain the best results in ADG and feed efficiency. According to Van Emon *et al.* (2012), lambs can be fed up to 50% DDGS without negative effects on feedlot performance. The authors suggest that lambs respond differently to increased dietary inclusion of DDGS compared to cattle. Schauer *et al.* (2008) reported that levels up to 22.5% in a finishing diet would have no negative effects on lamb performance or carcass traits. The current study had 23.8% of DDGS in the DDGS treatment while the OPT treatment contained only 4.8% of DDGS (Table 4.1), well within the recommendations found in the literature.

The more efficient a lamb is to convert feed to live weight gain (FCR), the more profitable the intensive lamb finishing enterprise would be (MLA, 2010). The higher the FCR value, the lower the efficiency of conversion of feed into an animal product (Wilkinson, 2011). The DDGS treatment diet had a mean FCR of 4.68 compared to CSM, which had the poorest FCR of 5.48 for the total experimental period (Table 4.6), but the difference was not significant. The effect of FCR throughout the whole experimental period can be seen in Table 4.5 and Figure 4.6. Significant variation in FCR between the CSM treatment lambs, which performed the poorest, ($P < 0.05$) compared to OPT fed lambs which performed the best, were observed in the first week. As the study progressed, (Table 4.5) until the last week, DDGS apart from OPT, were performing significantly better ($P < 0.05$) compared to all the other treatment diets.

Table 4.5 The weekly FCR of the lambs on different treatment diets over the experimental period of four weeks.

Item		Treatment[1]					
	Period[2]	DBG	DDGS	CSM	SBM	OPT	SEM
FCR	1	4.8^{ab}	5.0^{ab}	6.8^{a}	4.6^{ab}	3.7^{b}	1.05
	2	3.5	4.2	4.0	4.4	4.8	0.49
	3	8.8^{a}	8.2^{a}	7.5^{ab}	5.3^{b}	6.5^{ab}	1.39
	4	8.7^{a}	4.6^{b}	8.1^{a}	7.9^{a}	5.9^{ab}	1.05

[1]Treatment: Specific protein concentrate included in the experimental diet. SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, OPT= optimal diet DDGS = distillers' grains with solubles. [2]Periods were 1 week in length. Means within a row without a common superscript letter differ ($P < 0.05$).

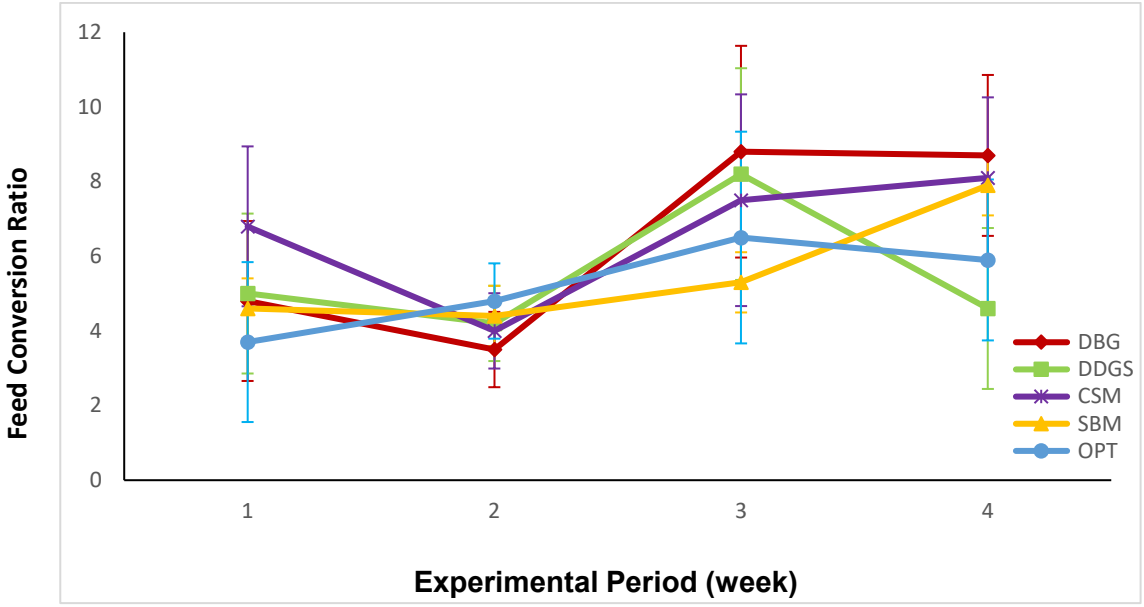


Figure 4.6 The FCR of the lambs on different treatment diets over the experimental period of 4 weeks. (Error bars represent the SE concerned).

The OPT treatment group performed very well overall in many of the growth performance parameters compared to the diets where the two by-products, DDGS and DBG, were fed separately. When single concentrate feeds are mixed forming compound feeds potential interactions (associative effects) might occur (Grubješić *et al.*, 2020). Associative effects can be defined as the sum of the parts being less, or more, than the combination of the parts (Robinson *et al.*, 2009). The better performance could have been attributed to the combination of protein sources used which had different rumen degradability in the OPT treatment, therefore stimulating the appetite and leading to partition in proteins between the microbes and the host itself (Faverdin, 1999). Faverdin (1999)

explained this phenomenon by suggesting an improvement in feed digestibility and microbial activity, because of better degradable crude protein in the rumen, faster and more complete digestion of the feed by microbes reduces the fill of the feed in the rumen, and thus leads to an increase in DMI. Another possible explanation of the higher DM intake of the animals fed the OPT diet compared to the DDGS diet by Huhtanen *et al.* (2012) is that there is an increased supply and/or more balanced supply of AA that improves the performance and as a result of increased energy demand DM intake is increased. Amino acid ratios are normally better balanced when more than one source is used (Wu *et al.*, 2014). However, the reasons for an associative effect are not clear (Robinson *et al.*, 2009) and more knowledge regarding the effects amongst the feeding values of single feeds in compound feeds is needed (Grubješić *et al.*, 2020).

According to Lima *et al.* (2017), lambs with the same DMI might have the same mean daily gain in diverse weights and therefore, generate different incomes. It is thus necessary to look at the feed efficiency of the lambs because this variable is strongly correlated with profitability (Nascimento, 2011). Final body weight, body weight change, daily body weight gain (ADG), total DMI, feed intake as a percentage of BW and feed conversion ratio (FCR) of lambs on the different experimental diets are presented in Table 4.6. No significant differences were observed for any of the above-named parameters among different treatment diets, except for total mean DMI. As presented in Table 4.6 the total DMI for the lambs fed DDGS were lower compared to the lambs fed the OPT diet. Possible reasons for this were already discussed in section 4.7.2.

Table 4. 6 Mean growth performance, feed intake and feed conversion of Dohne Merino lambs fed different experimental diets.

	Treatment						
	1	**2**	**3**	**4**	**5**		
Item	**DBG**	**DDGS**	**CSM**	**SBM**	**OPT**	**SEM**	***P*-value**
Growth Performance							
Initial live body weight (kg)	30.72	30.34	32.70	31.87	31.71	0.981	0.33
Final live body weight (kg)	39.64	39.62	41.63	41.09	42.18	1.050	0.32
Body weight gain (kg)	8.93	9.28	8.93	9.22	10.46	0.579	0.33
ADG (kg/day)	0.288	0.299	0.288	0.297	0.338	0.019	0.33
Total Feed Intake							
Feed intake (kg DM)	47.13^{ab}	43.37^{b}	46.99^{ab}	46.93^{ab}	50.15^{a}	1.634	0.10
Daily DMI							
Feed intake (% of BW)	4.33	4.00	4.09	4.18	4.38	0.151	0.36
Feed conversion Ratio							
FCR (DMI/kg gain)	5.39	4.68	5.48	5.12	4.91	0.33	0.40

[1]Treatment: Specific protein concentrate included in the experimental diet. SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, OPT= optimal diet DDGS = distillers' grains with solubles. Means within a row without a common superscript letter differ ($P < 0.05$).

According to the NRC, (1985) the daily dry matter intake of fast-growing lambs can vary between 3.8-4.2% of live weight. The mean for total DMI as a percentage of BW in the current study was 4.2% and did not differ among treatments. Final mean body weight was the highest for lambs fed the diets containing OPT, followed by CSM > SBM > DBG > DDGS.

Considering ruminants, a supply of amino acids can be obtained through rumen microbes (NRC, 2007). According to the NRC (2001), the AA profile of ruminal microbes and endogenous protein tends to be constant. It is therefore crucial to predict the AA composition of RUP because this will help to identify the limiting AA, which will lead to a more balanced diet for production. To determine the AA composition, samples should be incubated for 16 hours and be compared with the AA concentration of the original feed (Maxin *et al.,* 2013). Although the amino acid ratios of the diets were not measured, a constraint for optimal production in animals fed DDGS might be that the lysine content could have been deficient (Wadhwa & Bakshi, 2016; Chen *et al.*, 2021). In contrast, SBM is known to have a high content of lysine (Cromwell, 1999), but the SBM treatment did not manifest higher weight gains than other treatments. In a study conducted by Parsons *et al.* (1983), broiler chickens were fed DDGS or SBM as the sole source of dietary protein. In the latter study, the chicks that were fed SBM gained weight faster and were more efficiently ($P < 0.05$) compared to chicks fed DDGS. During the drying of distillers grains, heat damage could occur and specifically, lysine could be particularly susceptible to it due to its ε-amino group (Mauron, 1990).

DDGS drying may be beneficial to some extent by increasing RUP (Van Emon *et al.*, 2012). According to Van Emon *et al.* (2012), it is due to Maillard reactions by denaturing proteins and forming protein-carbohydrate and protein-protein cross-links. However, heating may also produce indigestible Maillard products (Nakamura *et al.*, 1994). Nevertheless, to optimize the production of e.g., meat, amino acids, which include lysine, may be limited to meet the protein synthesis requirement (NRC, 2007). In a study conducted by Han *et al.* (1996), three levels of rumen-protected lysine (RPL) were added at 0% (T1), 0.32% (T2) and 0.65% (T3) to concentrate diets. The live weight gains were greater ($P < 0.05$) for sheep fed the T3 diet than those fed the diet T1 or T2 (244 vs 219 or 216 g/d, respectively).

In another study done by (Chen *et al.*, 2021) five different diets were fed to lambs: (1) the control group (CON) which diet contained SBM, (2) the DDGS group (NSM), without any SBM and rumen-protected amino acids (RPAA), (3) the DDGS with RP lysine group (DRPL), (4) the DDGS with RP methionine group (DRPM), and (5) the DDGS with a mixture of RP lysine and methionine group (DRPLM). The four dietary treatments that contained DDGS were formulated to be similar to the crude protein (CP) level of the CON diet. The results indicated the final BW of the DRPLM group increased ($P < 0.05$) compared to NSM, DRPL and DRPM groups, but no difference was observed compared to CON.

4.7.5. **Carcass characteristics**

The protein sources of each treatment diet may affect the carcass characteristics and meat composition (Khalid *et al.*, 2012). According to Khalid *et al.* (2012), these may include warm carcass weight (WCW), cold carcass weight (CCW) and dressing percentage (DP). In the current study, no significant differences were observed among treatments (Table 4.7) for warm carcass weight, cold carcass weight and dressing percentage. It is important to take the energy density of the diet into account because it influences the carcass fatness (Khalid *et al.*, 2012). For instance, the treatment diet containing SBM, had a slightly higher energy content ME (MJ/kg) and starch content compared to the other treatment diets. Most lambs across all treatment diets were classified as class 2 of the carcass fat classification (Figure 4.7). All the lambs fed diets containing SBM had 100% of carcasses with fat class 2 compared to the other treatment diets which had 86-71% of carcasses with a fat class 2 and the rest of the carcasses with a fat class 1. When there is a large supply of lambs, A2 carcasses will be given the premium prices, while the price of A3 lamb will decrease accordingly (Van der Merwe *et al.*, 2020b).

Table 4.7 Carcass characteristics of Dohne Merino lambs fed different experimental diets.

	Treatment						
	1	**2**	**3**	**4**	**5**		
Item	**DBG**	**DDGS**	**CSM**	**SBM**	**OPT**	**SEM**	***P* - value**
WCW (kg)	18.87	18.67	19.75	19.75	20.07	0.582	0.37
CCW (kg)	18.30	18.11	19.16	19.16	19.47	0.564	0.38
Dressing % (CCW)	46.21	45.65	45.72	46.50	46.50	0.675	0.90

[1]Treatment: Specific protein concentrate included in the experimental diet. SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, OPT= optimal diet DDGS = distillers' grains with solubles. WCW = warm carcass weight, CCW = cold carcass weight.

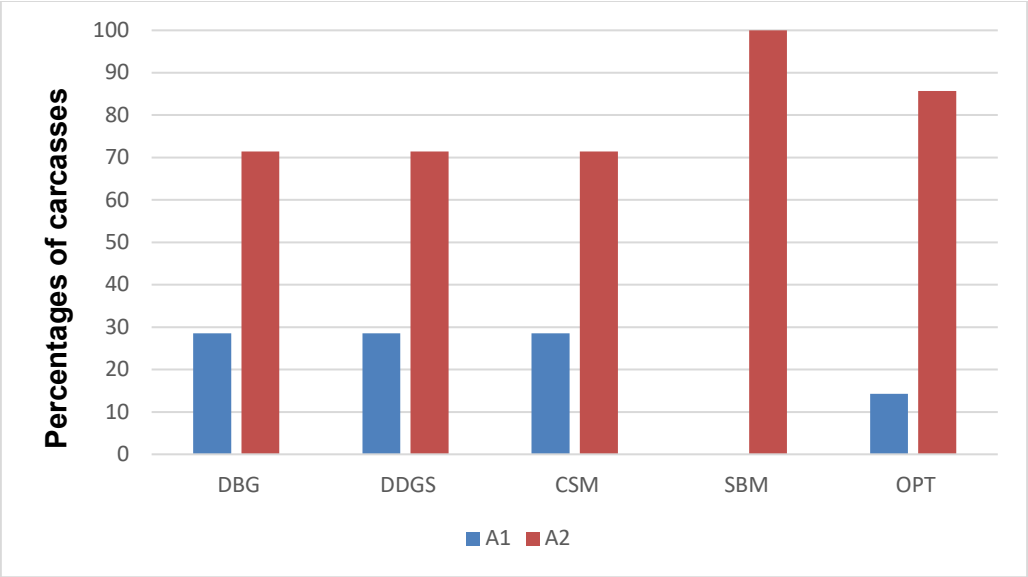


Figure 4.7 Carcass classification of feedlot lambs fed different treatment diets.

4.7.6. **Economic Analysis**

The profitability of a lamb feedlot system is dependent on adding value to the carcasses in the form of tissue growth to obtain a more desirable product (Van der Merwe, 2020), which meets market specifications with a higher economic value. The economic evaluation was done to the point of margin above specified cost, and was performed by deducting the initial live weight cost of the wether lambs and feed cost from the income of the carcasses without considering the other fixed and operational costs related to sheep production. A comparison of the cost and margin above specified costs is depicted in Table 4.8. The live lamb price was R 40/kg upon arrival at the experimental farm. The carcass price (R 83/kg) were estimated using the market prices on the day of slaughter (22 October 2021). The margin above specified costs were affected by the total DM feed intake as well as the feed price for each of the treatment groups. No significant differences were observed among the five treatment diets ($P > 0.05$) for margin above specified costs.

Table 4.8 A comparison of the allocatable cost and margin above specified costs for the five treatment groups.

	Treatment[1]						
	1	2	3	4	5		
Item	**DBG**	**DDGS**	**CSM**	**SBM**	**OPT**	**SEM**	***P* - value**
Initial weight (kg)	30.72	30.34	32.70	31.87	31.71	0.981	0.33
Lamb cost R/ lamb	1228.80	1213.44	1308.16	1274.88	1268.48	39.24	0.46
Feed intake (DM) (kg)	47.13^{ab}	43.37^{b}	46.99^{ab}	46.92^{ab}	50.15^{a}	1.634	0.10
Feed price (R/t)	2982.43	3015.45	3155.39	3314.39	2975.58	-	-
Feed cost (R/lamb)	156.48^{ab}	148.06^{b}	164.31^{a}	172.44^{a}	167.17^{a}	5.591	0.04
Total cost (R)	1385.26	1361.50	1472.47	1447.32	1435.65	40.52	0.30
CCW (kg)	18.30	18.11	19.16	19.16	19.47	0.564	0.38
Income (R/lamb)	1518.96	1502.85	1590.07	1590.10	1615.57	46.85	0.37
Margin above specified costs (R)	133.68	141.35	117.60	142.75	179.92	24.63	0.50

[1]Treatment: Specific protein concentrate included in the experimental diet. SBM = soybean meal, CSM = cottonseed meal, DBG = dried brewers grain, OPT= optimal diet DDGS = distillers' grains with solubles. CCW = cold carcass weight .Means within a row without a common superscript letter differ ($P < 0.05$).

4.8. CONCLUSION

The treatment diets containing different protein sources appeared to have limited effects on growth performance and were slaughtered at similar live weights when formulated to be iso-nitrogenous, and iso-energetic. No significant differences among the five treatment diets for final body weight, growth performance, carcass weights were observed as well as for margin above specified costs. The results of this study indicated that DDGS can substitute commonly used protein sources and a portion of maize used in lamb feedlot diets, without negatively affecting feedlot performance or carcass quality.

4.9. REFERENCES

Ahmad, S., Jabbar, M. A., Khalique, A., Saima, Shahzad, F., Ahmad, N., Fiaz, M., & Younas, U. 2014. Effect of different levels of NDF on voluntary feed intake, dry matter digestibility and nutrients utilization in dry nili ravi buffaloes. J. Anim. Plant Sci. 24, 1602–1605. Available at: http://www.thejaps.org.pk/docs/v-24-6/03.pdf.

Akhtar, M., Ali, M., Hayat, Z., Yaqoob, M., & Sarwar, M. 2016. Effect of varying levels of dietary ruminal undegradable protein on feed consumption and growth performance of growing Kajli lambs. Int. J. Agric. Biol. 18, 969–974. https://doi.org/10.17957/IJAB/15.0196.

AOAC International. 2002. Official Method of Analysis. 17th ed. Association of Official Analytical Chemists Inc., Arlington, Virginia, USA.

Beauchemin, K. A., McClelland, L. A., Kozub, G. C., & Jones, S. D. M. 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. https://doi.org/10.4141/cjas95-058.

Brand, T. S., & van der Merwe, G. D. 1993. Comparison of different protein sources in enriched grain mixture for fattening lambs. S. Afr. J. Anim. Sci. 23, 13–17. Available at: https://journals.co.za/doi/pdf/10.10520/EJC-1eb29164c1.

Cannas, A., Tedeschi, L. O., Fox, D. G., Pell, A. N., & Van Soest, P. J. 2004. A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. J. Anim. Sci. 82, 149–169. https://doi.org/10.2527/2004.821149x.

Chen, J., Niu, X., Li, F., Li, F., & Guo, L. 2021. Replacing soybean meal with distillers dried grains with solubles plus rumen-protected lysine and methionine: Effects on growth performance, nutrients digestion, rumen fermentation, and serum parameters in Hu sheep. Animals 11, 1–11. https://doi.org/10.3390/ani11082428.

Chipa, M. J., Siebrits, F. K., Ratsaka, M. M., Leeuw, K. J., & Nkosi, B. D. 2010. Growth performance of feedlot weaners cattle fed diet containing different levels of cold press soya bean oilcake. South African J. Anim. Sci. 40(5), 499–501. Available at: https://www.sasas.co.za/wp-content/uploads/2012/09/Chipa40Issue5Congress_0.pdf.

Cromwell, G. L. 1999. Soybean Meal - The " Gold Standard ". Farmer's Pride, KPPA News 11, 9–11.

DAFF. 2020. South African Feed Market Analysis Report.

De Jager, W. 2016. Forecast estimates of protein for animals in South Africa. MSc Thesis. University

of the Free State, Bloemfontein, South Africa.

Eliasson, A. C. 1981. On the possibility of modifying the gelatinization properties of starch by lipid surface coating. Stärke 33 (7): 231 - 235. https://doi.org/10.1002/star.19810330704.

Faverdin, P. 1999. The effect of nutrients on feed intake in ruminants. Proc. Nutr. Soc. 58, 523–531. https://doi.org/10.1017/S0029665199000695.

Ferret, A., Calsamiglia, S., Bach, A., Devant, M., Fernández, C., & García-Rebollar, P. 2008. Necesidades Nutricionales Para Rumiantes de Cebo Normas.in Fundación Española para el Desarrollo de la Nutrición Animal. Madrid.

Forbes, J. M. 2007. Voluntary Food Intake and Diet Selection in Farm Animals, 2nd edn.

Grimsell, A. 2020. Effect of protein source and quality on feedlot growth performance and rumen fermentation characteristics of Döhne Merino lambs. MSc Thesis. University of Pretoria, Pretoria, South Africa.

Grubješić, G., Titze, N., Krieg, J., & Rodehutscord, M. 2020. Ruminal fermentation characteristics and related feeding values of compound feeds and their constituting single feeds studied by using *in vitro* techniques. Animal 14, 1829–1840 https://doi.org/10.1017/S1751731120000889.

Gurung, N. K., Solaiman, S. G., Rankins, D. L., & McElhenney, W. H. 2009. Effects of distillers dried grains with solubles on feed intake, growth performance, gain efficiency and carcass quality of growing Kiko x Spanish male goats. J. Anim. Vet. Adv. 8(10), 2087–2093. https://doi.org/10.1080/09712119.2011.640205.

Haddad, S. G., Kridli, R. T., & Al-Wadi, D. M. 2005. Influence of varying levels of dietary undegraded intake protein intake on nutrient intake, body weight change and reproductive parameters in postpartum Awassi ewes. Asian-Australasian J. Anim. Sci. 18, 637–642. https://doi.org/10.5713/ajas.2005.637.

Hall, M. B. 2009. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. J AOAC Int 92, 42–49.

Han, I. K., Ha, J. K., Lee, S. S., Ko, Y. G., & Lee, H. S. 1996. Effect of supplementing rumen-protected lysine on growth performance and plasma amino acid concentrations in sheep. Asian-Australasian J. Anim. Sci. 9, 309–313. https://doi.org/10.5713/ajas.1996.309.

Hassan, S. A., & Bryant, M. J. 1986. The response of store lambs to dietary supplements of fish meal. Anim. Prod. 42(2), 233–240. doi:10.1017/S0003356100017943.

Hornick, J. L., Van Eenaeme, C., Gérard, O., Dufrasne, I., & Istasse, L. 2000. Mechanisms of reduced and compensatory growth, Domestic Animal Endocrinology. Domest. Anim.

Endocrinol. 19(2),121-32. DOI: 10.1016/s0739-7240(00)00072-2.

Huhtanen, P., Hetta, M., & Swensson, C. 2012. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. Can. J. Anim. Sci. 91, 529–543. https://doi.org/10.4141/cjas2011-029.

Ikram, S., Huang, L. Y., Zhang, H., Wang, J., & Yin, M. 2017. Composition and Nutrient Value Proposition of Brewers Spent Grain. J. Food Sci. 82, 2232–2242. https://doi.org/10.1111/1750-3841.13794.

Indexmundi. 2022. Soybean meal - Monthly price (r/metric ton). Accessed April 2022. https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=240&currency=zar.

Jafarnejad, S., Farkhoy, M., Sadegh, M., & Bahonar, A. R. 2010. Effect of crumble-pellet and mash diets with different levels of dietary protein and energy on the performance of broilers at the end of the third week. Vet. Med. Int. 2010, Article ID 328123. https://doi.org/10.4061/2010/328123.

Khalid, M. F., Sarwar, M., Rehman, A. U., Shahzad, M. A., & Mukhtar, N. 2012. Effect of Dietary Protein Sources on Lamb's Performance: A Review. Iran. J. Appl. Anim. Sci. 2, 111–120. Available at: https://ijas.rasht.iau.ir/article_513536_f03e95beedc67416a3ad54da123a1ad1.pdf.

Kirk, W. G., Chapman, H. L., Peacock, F. M., & Davis, G. K. 1969. Utilizing bagasse in cattle fattening rations. University of Florida Exp. Sta. Bull. 641 A.

Klopfenstein, T. J., Erickson, G. E., & Bremer, V. R. 2008. BOARD-INVITED REVIEW: Use of distillers by-products in the beef cattle feeding industry. J Anim Sci 86, 1223–1231. https://doi.org/doi: 10.2527/jas.2007-0550.

Leng, R. A., Hillard, M. A., & Nolan, J. V. 1981. Mechanism of action of bypass proteins. Conference Paper. Available at: https://www.researchgate.net/publication/279986091_MECHANISM_OF_ACTION_OF_BYPASS_PROTEINS.

Li, B., Sun, X., Huo, Q., Zhang, G., Wu, T., You, P., He, Y., Tian, W., Li, R., Li, C., Li, J., Wang, C., & Song, B. 2021. Pelleting of a Total Mixed Ration Affects Growth Performance of Fattening Lambs. Front. Vet. Sci. 12(8), 629016. https://doi.org/10.3389/fvets.2021.629016.

Lima, N. L. L., de Freitas Ribeiro, C. R. R., de Sa, H. C. M., Leopoldino-Júnior, I., Cavalcanti, L. F. L., Santana, R. A. V., Furusho-Garcia, I. F., & Pereira, I. G. 2017. Economic analysis, performance, and feed efficiency in feedlot lambs. Rev. Bras. Zootec. 46, 821–829. https://doi.org/10.1590/S1806-92902017001000005.

Mabele Fuels. 2021. http://www.mabelefuels.com/products-services/ddgs/.

Mahyuddin, P. 2009. Compensatory Growth in Ruminants. pp 125–134. Anim. Prod. 6(2), 125-135. Avaialbel at: http://www.animalproduction.net › article › download.

Malik, R., Razzaque, S., Al-Khozam, N., & Sahni, S. 1996. 'Feedlot Growth & Efficiency of Three-Way Cross Lambs as Affected by Genotype, Age & Diet. Anim. Prod. 21, 251–254.

Mauron, J. 1990. Influence of processing on protein quality. J Nutr Sci Vitaminol 36, 57–69. DOI: 10.3177/jnsv.36.4-supplementi_s57.

Maxin, G., Ouellet, D. R., & Lapierre, H. 2013. Ruminal degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distillers grains. J. Dairy Sci. 96, 5151-5160.

McDonald, P., Edwards, R., Edwards, R. A., Greenhalgh, J. F. D., & Morgan, C. A. 2002. Animal Nutrition. 6th ed. Pearson, Essex, England.

MLA. 2007. A producers' guide to production feeding for lamb growth. Meat & Livestock Australia Limited.

MLA. 2010. Best practice for production feeding of lambs: A review of the literature (S Jolly, Ed.). Meat & Livestock Australia Limited.

Morrison, S. R. 1983. Ruminant heat stress: effect on production and means of alleviation. J. Anim. Sci. 57, 1594–1600. DOI: 10.2527/jas1983.5761594x.

Muela, E., Sañudo, C., Campo, M. M., Medel, I., & Beltrán, J. A. 2010. Effects of cooling temperature and hot carcass weight on the quality of lamb. Meat Sci. 84, 101–107. https://doi.org/10.1016/j.meatsci.2009.08.020.

Nakamura, T., Klopfenstein, T. J., Gibb, D. J., & Britton, R. . 1994. Growth efficiency and digestibility of heated protein fed to growing ruminants. J. Anim. Sci. 72, 774–782. DOI: 10.2527/1994.723774x.

Nascimento, M. L. 2011. Eficiência alimentar e suas associações com lucro, características de carcaça e qualidade de carne de bovinos Nelore. PhD thesis, University of São Paulo, Piracicaba, Brazil.

NRC. 1985. Nutrient requirements of sheep (National Research Council, Ed.). 6th ed. Washington, D.C.

NRC. 2001. Nutrient Requirements of Dairy Cattle. seventh. The National Academies Press, Washington DC.

NRC. 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World

Camelids. The National Academies Press, Washington DC.

Oliveira, M. A., Alves, S. P., Santos-Silva, J., & Bessa, R. J. B. 2017. Effect of dietary starch level and its rumen degradability on lamb meat fatty acid composition. Meat Sci. 123, 166–172. https://doi.org/10.1016/j.meatsci.2016.10.001.

Parsons, C. M., Baker, D. H., & Harter, J. M. 1983. Distillers Dried Grains with Solubles as a Protein Source for the Chick. Poult. Sci. 62, 2445–2451. https://doi.org/10.3382/ps.0622445.

Preston, T. R., & Leng, R. A. 1987. Matching ruminant production systems with available resources in the tropics and sub-tropics. Penambul books, Australia, pp 265.

Robinson, P. H., Getachew, G., & Cone, J. W. 2009. Evaluation of the extent of associative effects of two groups of four feeds using an in vitro gas production procedure. Anim. Feed Sci. Technol. 150, 9–17. https://doi.org/10.1016/j.anifeedsci.2008.07.010.

SAPPO. 2021. Raw material statistics, South African Pork Industry. https://sappo.org/.

Schauer, C. S., Stamm, M. M., Maddock, T. D., & Berg, P. B. 2008. Feeding of DDGS in lamb rations. Sheep Goat Res. J. 23, 15–19. Available at: https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/ba3468a2a8681f69872569d60073fde1/5703a371897ed680872575d200764f58/$FILE/Feeding_of_DDGS_in_lamb_rations.pdf.

Seo, S., Jeon, S., & Ha, J. K. 2018. Guidelines for experimental design and statistical analyses in animal studies submitted for publication in the Asian-Australasian Journal of Animal Sciences. Asian-Australasian J. Anim. Sci. 31, 1381–1386. https://doi.org/10.5713/ajas.18.0468.

Sheridan, R., Ferreira, A. V, & Hoffman, L. C. 2003. Production efficiency of South African Mutton Merino lambs and Boer goat kids receiving either a low or a high energy feedlot diet. Small Rumin. Res. 50, 75–82. https://doi.org/10.1016/S0921-4488(03)00109-3.

Silva Sobrinho, A. G. 2001. General aspects under production sheeps carcass. Rev. Electrón. vet. 17, 425-453. Available at: https://www.redalyc.org/pdf/636/63647454002.pdf.

Smith, P. S. 1983. Food starches and their uses. In: D.L. Downing (Editor) Gum and Starch Technology Eighteenth Annual Symposium. New York State Agricultural Experiment Station, Geneva. 1 - 9.

South African Government. 2000. Meat Safety Act | South African Government. Cape Town.

STATISTICA 14. 2020. TIBCO Software Inc. Data Science Workbench, version 14. http://tibco.com.

Van der Merwe, D. A. 2020. Modelling the growth, feed intake and backfat deposition of different South African sheep breed types. PhD thesis, University of Stellenbosch, Stellenbosch, South Africa.

Van der Merwe, D. A., Brand, T. S., & Hoffman, L. C. 2020a. Premium lamb production of different South African sheep breed types under feedlot conditions. S. Afr. J. Anim. Sci., 1–12. http://dx.doi.org/10.4314/sajas.v50i4.9.

Van der Merwe, D. A., Brand, T. S., & Hoffman, L. C. 2020b. Precision finishing of South African lambs in feedlots: a review. Tropical Animal Health and Production 52, 2769-2786

Van Emon, M. L., Gunn, P. J., Neary, M. K., Lemenager, R. P., Schultz, A. F., & Lake, S. L. 2012. Effects of added protein and dietary fat on lamb performance and carcass characteristics when fed differing levels of dried distiller's grains with solubles. Small Rumin. Res. 103, 164–168. https://doi.org/10.1016/j.smallrumres.2011.09.002.

Van Niekerk, B. D. H. 1981. Byproducts of the sugar industry as animal feeds. S. Afr. J. Anim. Sci. 11, 119–137. Available at: https://journals.co.za/doi/pdf/10.10520/EJC-1fcfb887f2.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74, 3583–3597. DOI: 10.3168/jds.S0022-0302(91)78551-2.

Wadhwa, M., & Bakshi, M. P. S. 2016. Application of Waste-Derived Proteins in the Animal Feed Industry.Pages 161–192 in Protein Byproducts: Transformation from Environmental Burden Into Value-Added Products. Academic Press.

Warriss, P. D. 2000. Meat Science. CABI Publish, New York, USA.

Wilkinson, J. M. 2011. Re-defining efficiency of feed use by livestock. Animal. 5(7), 1014–1022. DOI: 10.1017/S175173111100005X.

Wu, G., Bazer, F. W., Dai, Z., Li, D., Wang, J., & Wu, Z. 2014. Amino acid nutrition in animals: protein synthesis and beyond. Annu Rev Anim Biosci. 2, 387–417. https://doi.org/10.1146/annurev-animal-022513-114113.

Yildiz, E., & Todorov, N. 2014. The comparison of the main protein sources for dairy cows. A review. Bulg. J. Agric. Sci. 20, 428–446. Available at: http://agrojournal.org/20/02-28.pdf.

Zhang, X., Li, G., Li, F., Zhang, D., Yuan, L., Zhao, Y., Zhang, Y., Li, X., Song, Q., & Wang, W. 2021. Effect of feed efficiency on growth performance, body composition, and fat deposition in growing Hu lambs. Anim. Biotechnol. https://doi.org/10.1080/10495398.2021.1951747.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

Large parts of South African agricultural land is suitable for extensive livestock farming. However, this type of meat production system might not be sufficient and sustainable to meet the increasing demands of a growing human population. In addition, many external factors including droughts, stock theft, predatory losses and disease outbreaks affects sustainability and profitability of extensive livestock farming. Intensive finishing systems could however mitigate these challenges.

With intensively reared livestock, high nutrient-density diets are fed which include cereal grains and protein feeds, including soybean meal (SBM), to ensure high feed efficiency. Protein supplements are a very costly component of the diet and by-products, such as dried distiller grains with solubles (DDGS) could be an effective substitute to more expensive protein raw materials. This study investigated the potential of DDGS to replace SBM in comparison with other protein raw materials, dried brewer grains (DBG), cottonseed meal (CSM), and a least cost optimal formulated diet of these mentioned protein sources (OPT) for growing lamb diets.

Chapter 3 focused on the extent and rate of *in vitro* ruminal protein degradation of the different protein sources, namely soybean meal (SBM), cottonseed meal (CSM), dried brewers grain (DBG) and canola meal (CM) against DDGS for ruminants. This information was critical for the optimal formulation of diets used in the growth trail because feed ingredients with a low rumen degradability could increase the bypass nutrients allowing small intestinal digestibility. *In vitro* ruminal crude protein degradation with a passage rate (k_p) of 5% and 8%/h was determined in an Ankom Daisy^{II} incubator. The soluble fraction (a) was the highest for DDGS (25.3%) followed by CM (21.2%) and SBM (19.4%). The lowest concentrations of soluble CP (fraction a) were observed in DBG (13.6%) and CSM (14.5%). For fraction (b), the fraction that would potentially be degraded over time, SBM (62.0%) had the highest value and CSM (37.7%) had the lowest. No significant variation between the feeds for the rate of degradation of fraction b was observed. The *in vitro* analysis indicated that SBM was more rumen degradable than DDGS. The estimated RUP fraction for the different protein sources, assuming k_p = 8%/h, was 46.7% for SBM; 49.2% for CM; 49.7% for DDGS; 64.3% for CSM and 68.1% for DBG, respectively. The results of the study supported the sequence in which protein degradation occurred among the respective protein sources, as reported by other studies, except for SBM which was more degradable in the rumen than CM. The results from this study would contribute to effective ruminant diet formulation. There is little known data on rumen degradability and variability

of dried distillers' by-products produced in South Africa. High variability often complicates accurate diet formulation with DDGS.

In Chapter 4, the different protein sources (SBM, DDGS, DBG CSM, and a least cost optimal formulated diet of these mentioned protein sources (OPT) were compared in a growth trial where growth parameters, carcass characteristics and economical parameters of feedlot lambs were determined. The results indicated no differences among the treatment diets for the final lamb live weights, average daily gain (ADG) and feed conversion ratio (FCR) as well as for margin above specified costs at the end of the growth trail.

The use of DDGS in growing lamb feed rations needs adequate and accurate information among feed formulators regarding the characteristics, parameters, value, and other aspects of DDGS. The results of this study contribute to the limited knowledge of the nutritional value of South African-produced DDGS as a protein source in lamb feedlot diets in South Africa. DDGS further has the potential to lower the feed cost, thereby improving the economical production of feedlot lamb carcases. These findings may also assist in creating a potential market for locally produced biofuel by-products.

In conclusion, these findings support the hypothesis that production responses of feedlot lambs would be similar when fed rations formulated with different protein sources, provided their protein, energy, vitamin, and mineral requirements are being met. It can be concluded that the results of this study prove that DDGS, CSM and DBG can replace SBM in lamb-growing diets without having a negative impact on ADG or feed efficiency.

5.2. RECOMMENDATIONS

Given the high variability of dried distillers' by-products in South Africa, more *in vitro* studies should be done to compare the rate and extent of protein degradation with conventional protein sources. Standardization of the production process of DDGS will contribute to a decrease in variability. The OPT treatment group performed very well overall in many of the growth performance parameters compared to the diets where the two by-products, DDGS and DBG, were fed separately. This could be due to a better balance between amino acid (AA) and energy absorbed. Therefore, further studies should be done on the AA composition of the protein supplements remaining after 16 h of ruminal incubation and the 16-h AA concentration to original feed AA concentration ratio.