Effect of protein source and level of urea supplementation on performance and rumen fermentation dynamics in feedlot lambs

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by

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Submitted in partial fulfillment of the requirements for the degree

M.Sc. (Agric): Animal Science: Animal Nutrition

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Declaration

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

Signature: …….

Date: …….

I.H. Greeff

Pretoria

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Summary

Effect of level of urea supplementation on performance and rumen fermentation dynamics in feedlot lambs

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In the intensive livestock industry, protein is commonly the most expensive feed component. Urea can be included in ruminant diets as an economical replacement for part of the natural protein. Two experiments were conducted in which animals received diets with different levels of urea. The treatments were 0% urea (control), 0.42% urea, 0.83% urea, 1.25% urea and 1.66% urea. Diets were fed as TMR's and formulated to contain equal amounts of energy, CP, and NDF. Experiment 1, a randomized complete block design with 250 South African Mutton Merino lambs, was conducted to assess the effects of different inclusion levels of urea on feed intake, growth performance, and carcass characteristics of lambs under practical feedlot conditions. Results from experiment 1 showed that lambs fed the 1.66% urea diet had a lower ($P < 0.05$) DMI compared with the 0% and 0.42% urea treatments, and there was a linear decrease ($P < 0.05$) in DMI as the levels of urea increased. The 0% treatment and 1.66% urea treatment had a better FCR ($P < 0.05$) than the 0.83% urea treatment, and there was a quadratic relationship ($P < 0.05$) between FCRand urea inclusion levels. The 0% urea treatment had a higher ADG ($P < 0.05$) than the 0.83% and 1.25% urea treatment. The 0% and 0.42% urea treatments had a higher ($P < 0.05$) cold carcass weight compared with the 0.83%, 1.25% and

1.66% urea treatments. The 0.42% urea treatment tended to have a higher dressing percentagecompared with the 0.83% and 1.66% urea treatments.

Experiment 2, a 5 x 5 Latin square design with five rumen cannulated Merino wethers, investigated the effect of different inclusion levels of urea on rumen fermentation dynamics and apparent total tract digestibility. In contrast with the feedlot trial, DMI did not differ ($P > 0.05$) between treatments. The DM, OM, CP, and energy apparent digestibility coefficients were lower ($P < 0.05$) for the 0% urea compared with the 1.66% urea. An increase in apparent digestibility coefficients for DM, OM, CP, and energy was detected as the inclusion levels of urea was increased, however, this was probably due to the changes in the dietary ingredient composition,rather than a direct effect of increased urea levels since minimum NH_3-N level required was met for sheep on all the treatments. No differences ($P > 0.05$) were detected for starch digestibility. The digestibility of NDF tended to be lower for the 1.25% urea treatment compared with the 0% urea treatment.

The average rumen pH was higher ($P < 0.05$) for the 0% urea compared with the 1.25% urea treatment, and there was a quadratic relationship ($P < 0.05$) between average rumen pH and urea inclusion levels. The 0% urea treatment had a higher minimum pH ($P < 0.05$) than the 1.25% urea treatment. The 0% urea treatment had a higher maximum pH ($P < 0.05$) than the 0.83%, 1.25% and 1.66% urea treatments and there was a quadratic relationship ($P < 0.05$) between maximum rumen pH and urea inclusion levels. The average rumen NH_3-N was lower ($P < 0.05$) for the 0% urea compared with the 0.83%, 1.25% and 1.66% urea treatments. The minimum rumen NH_3-N was lower ($P < 0.05$) for the 0% urea treatment than the 0.83% treatment. The 0% urea treatment had a lower maximum rumen NH_3-N ($P < 0.05$) than the 0.83%, 1.25%, and 1.66% urea treatments, and there was a quadratic relationship between maximum NH_3-N and urea inclusion levels. The results suggest that urea can be included up to 1.66% of total diet DM in finishing lamb diets without having a depressed DMI, growth performance, digestibility, and fermentation of certain rumenparameters of sheep.

Keywords: average daily gain, digestibility coefficients, dry matter intake, feed conversion, rumen ammonia nitrogen, rumen pH.

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List of Abbreviations

AA	amino acid	mg	milligram
AUC	area under curve	MJ	Mega joules
ADFI	average daily feed intake	mL	milliliter
ADG	average daily gain	N	nitrogen
ADIN	acid detergent insoluble nitrogen	Na	sodium
^{o}C	degrees Celsius	NBPT	N-(n-butyl) thiophosphoric triamide
CSOCM	cottonseed oilcake meal	NDF	neutral detergent fibre
CP	crude protein	NH_3-N	ammonia nitrogen
DIP	degradable intake protein	NPN	non-protein nitrogen
DM	dry matter	NRC	National Research Council
DMD	dry matter digestibility	OM	organic matter
DMI	dry matter intake	OMD	organic matter digestibility
DOMI	digestible organic matter intake	OMI	organic matter intake
EE	ether extract	ppm	parts per million
FE	feed efficiency	RAN	rumen ammonia nitrogen
FME	fermentable metabolisable energy	RDP	rumen degradable protein
FCR	feed conversion ratio	RUP	rumen undegradable protein
FI	feed intake	SAMM	South African Mutton Merino

g	gram	SAS	statistical analysis system
GE	gross energy	SE	standard error
GIT	gastro intestinal tract	TDN	total digestible nutrients
H_2SO_4	sulphuric acid	TMR	total mixed ration
IVOMD	*In vitro* organic matter digestibility	UDP	undegradable dietary protein
kg	kilogram	USA	United States of America
L	liter	VFA(s)	volatile fatty acid(s)
ME	metabolisable energy		
m	meter		

Chapter 1

Introduction

In the intensive livestock industry, protein is commonly the most expensive feed component (Tao *et al.*, 2017). Urea can be fed to ruminants as an economical replacement for part of the crude protein (CP) in a ration. Urea is categorized under a class of feed nutrients named non-protein nitrogen (NPN), which contains no energy or rumen-undegradable protein (UDP). To meet the requirements of rumen degradable protein (RDP) in finishing diets for ruminants, it is common to replace some natural protein sources with urea (Akay *et al*., 2004). Urea is hydrolyzed by the action of urease synthesized by rumen bacteria, producing ammonia which is converted into microbial protein provided that sufficient fermentable metabolizable energy (FME) is available (Harmeyer & Martens, 1980). It has been suggested that the rapid hydrolysis of urea may negatively affect the efficiency of nitrogen (N) utilization in therumen and that it may also be toxic in excess (Bartley & Deyoe, 1981).

There is a perception that protein quality is not important in the finishing diets of lambs. Sindt *et al*. (1994) demonstrated that weaners on a finishing diet supplemented with soy-bean meal, gained faster and more efficient during the first 32 days than calves supplemented with urea and feather meal, but over the entire study (117 days) weight gain and efficiency were not affected by treatment. The response to a good quality protein in the early finishing period is made up by compensatory growth in the later stages of finishing (Bohman & Torell, 1956). Natural protein sources such as cottonseed meal and brewers-grains should contribute to better performance than alternative protein sources such as urea (Stanton & LeValley, 2014) since these sources also contain considerable amounts of UDP. The effect of protein sources on lamb performance was evaluated by Huston & Shelton (1971). They found that cottonseed oilcake meal (CSOCM) supplementation resulted in a higher average daily gain (ADG) and dry matter intake (DMI) and better feed efficiency (FE) when compared with lambs supplemented with urea.

Urea is one of the lowest cost-per-unit of protein values and therefore it is usually included in finishing ruminant diets (Stanton & LeValley, 2014). Urea contains about 45% N and protein contains 16% N, therefore when urea is converted to protein the CP equivalent value is approximately 281%. The inclusion of urea at 1% of the diet will increase CP content by 2.5%, however, it should not contribute more than 25% of total dietary CP and it should not be included at levels higher than 3% of totalDMI to prevent urea poisoning. It is also not recommended to feed urea to young lambs (< 30 kg) because their rumens are not fully developed and unable to efficiently utilize the NPN from urea (Duddy *et al*., 2016).

There is controversy with regards to the use of urea in young lambs. Some publications indicate that urea should not be included in the diets of young lambs (Willman *et al.,* 1946*;* Duddy *et al.,* 2016), while others indicate it does not affect production possibly due to compensatory growth at the later stages of development (Sindt *et al.,* 1994). Most studies, however, have been conducted on feedlot cattle and not finishing lambs (Sindt *et al*., 1994; Vander Pol *et al.,* 2006). There is therefore limited information on the importance of protein quality in feedlot lamb diets and the extent to which urea can replace natural plant-based protein sources such as CSOCM and further research is needed.

The objective of this study is to investigate to what extent urea can replace a high-quality natural protein source (CSOCM) in lamb feedlot diets and how it impacts rumen fermentation dynamics.

Chapter 2

Review of Literature

1. Introduction

The intensive feeding of cattle and sheep commonly referred to as feedlotting is a common practice throughout the world. The primary goal of any feedlot system is the same, to maximize the efficiency of growth and to minimize the cost per kilogram carcass gain. The objective when meeting the protein requirements of ruminants is to optimize the contributions of both microbial protein and dietary protein that escape ruminal degradation to supply amino acids available for absorption. The high cost of feed grains and many high-protein feedstuffs resulted in the optimum utilization of urea as a cost-effective feed ingredient in many feedlot diets. It's important to realize that NPN compounds are common constituents of the biological fluids of ruminants, even on an NPN-free diet. Several issues must be considered, though, to make its use most effective. The degradation of high-quality feed proteins to NH_3 in the rumen and then the re-synthesis of microbial protein from NH_3 is an inefficient process, and therefore the supply of RDP from NPN for ruminal microbial protein synthesis may be more economical than using natural protein sources (Shain *et al.,* 1998). However, Bolukbasi (1989) suggested that excess urea could decrease the reduction of nitrite to ammonia, thereby allowing an accumulation of nitrite in the rumen.

This chapter will be an overview of urea with emphasis on the usage of urea in ruminant nutrition, especially feedlot diets.

2. Overview of urea

2.1 Importance and use of urea

2.1.1 Brief history

Zuntz (1891, cited by Loosi *et al.,* 1949) proposed a theory that ruminal bacteria could utilize simple nitrogen components such as amides or ammonia to produce a bacterial protein which is then digested in the small intestine. During the First World War, a scarcity of plant protein developed in Germany, which stimulated research in the synthesis of urea and its use in ruminant nutrition (Krebs, 1937). At the same time research in the United Kingdom and the United States provided evidence that urea can effectively replace part of the natural plant protein in ruminant diets.

Bartlett and Cotton (1938) in the UK reported that when urea was used as a protein source in the diet of young cattle, satisfactory growth occurred. Hart *et al.* (1939) found that urea or ammonium bicarbonate can replace part of the natural plant protein in growing cattle and yield muscle tissue with normal protein content.

The value of urea for dairy heifers was also demonstrated by Work and Henke (1939) in Hawaii.

During the Second World War, the United States experienced a critical shortage of plant protein in the early 1940s, which stimulated the widespread use of urea in cattle and sheep diets. Improper mechanical mixing at that time, however, resulted inthe death of animals due to urea toxicity. In a study reported by Loosli *et al.* (1949), itwas demonstrated that urea could serve as the sole dietary nitrogen source for lambs. These researchers found that the 10 most essential dietary AA of the laboratory rat were synthesized in the rumen. Using the purified diet approach, they proved that lambs grew and remained in a positive nitrogen balance when offered such diets. Urea was approved in the USA as a ruminant feed ingredient in 1940 by the Association of American Feed Control Officials, but it was only in the 1950s that urea became a generally accepted ingredient in the diet of ruminants.

2.1.2 Global usage of urea

Urea is one of the most important and most popular chemicals in use today - as a fertilizer and as an industrial raw material. More than 80% of urea produced are used for fertilizer and more than 40% of all food grown in the world are fertilized by urea (Yamaguchi, 2017). Non-fertilizer applications can be used in the manufacture of urea-formaldehyde resins produced by the condensation reaction between urea and formaldehyde. These resins find outlets in adhesives for paperboard, plywood, surface coatings, molding resins, and textile processing. Another outlet is the synthesis of melamine, which is used for the production of melamine-formaldehyde resins. These are used in adhesives and paints, and for laminates, molding compounds, containers, and textiles. Another growing use for urea is in a process called selective catalytic reduction. It reduces nitrogen oxide emissions from diesel engines. This particular application for urea, while small relative to fertilizers, is the most rapidly growing end-use for urea, driven by air pollution regulations, originally in Europe, but expanding worldwide. Urea is also a component of ruminant diets. Urea has been one of the most important non-protein nitrogen supplements consumed by the animal feed industry.

By 1959 the world's industrial capacity to produce urea was estimated to be around 1,91 million metric tons (Hodges, 1965) compared with 171 million metric tonsin 2016 (Yamaguchi, 2017). China has been the world's largest urea producer. China,together with India and the Middle East, accounts for around two-thirds of the world's production of urea. China is the world's largest urea-consuming country, andtogether with India, China accounts for half of the world's consumption of urea. TheMiddle East, China, and Eastern Europe are the three largest exporters, accounting

for an estimated 76% of the world's urea exports. The top three regions accounted for an estimated half of the world's urea imports. The largest importing region is Southwest Asia, India accounted at about 22%, followed by South and Central Americas and the United States.

2.2 Utilisation of urea in ruminants

2.2.1 Mechanism of urea utilisation

The ability of ruminants to synthesize microbial protein in the rumen from NPN as well as from dietary protein sources complicates their protein and nitrogen metabolism. When urea enters the rumen as feed, it is rapidly dissolved and hydrolyzed by bacterial urease (Loosli & McDonald, 1968). Ammonia is the common denominator in the utilization of NPN by ruminants (Hungate, 1966). Unlike ruminal bacteria, protozoa are unable to convert urea or other NPN to protein because they lack the enzyme urease and therefore cannot utilize urea to synthesize amino acids (AA) needed for their growth (Jouany *et al.,* 1988). Marked changes have been observed in the rumen microbial population, in ruminants that had been fed purified and semi-purified diets without natural protein and NPN as their only N source (Dennis *et al.,* 1983). Nour *et al.* (1979) reported a decrease in the growth rate of protozoa *in vitro* and an increase in production time within the rumen when urea N inclusion levels were increased in the diet.

A close relationship exists between rumen microbial protein synthesis and those organisms breaking down cellulose and other carbohydrate materials (Loosli & McDonald, 1968). Carbohydrate breakdown by microbial enzymes produces volatile fatty acids (VFA) and keto acids. The breakdown of urea usually occurs at a faster rate than that of carbohydrates, especially if the diet has a high lignocellulose content. In such cases, the keto acids necessary for amino acid synthesis become limiting, and this results in a considerable loss of ammonia through the rumen wall. Toincrease the efficiency of ammonia utilization, the rate of carbohydrate hydrolysis and supply of keto acids need to be increased by supplying a source of energy that is rapidly fermentable. If the rate of urea intake is reduced to conform to the rate of cellulose hydrolysis on poor quality forages, the efficiency of nitrogen utilization can also be improved (Campling *et al.,* 1962).

The solubility of natural proteins varies greatly and thus the rate at which they are hydrolyzed and utilized by bacteria differs appreciably. However, a fairly high proportion of the more soluble proteins such as casein is utilized by bacteria in the same way as the ammonia from urea (Loosli & McDonald, 1968). When ammonia is produced too rapidly in the rumen or if the concentration becomes excessively high, considerable amounts are absorbed directly into the bloodstream, reconverted to

urea in the liver, excreted through the kidneys in the urine, and thus lost from the animal. Urea not excreted into the urine has two potential fates: (1) partitioned to the rumen where it is rapidly hydrolyzed to ammonia and then either used by bacteria for protein synthesis or absorbed into the blood, and (2) partition to the lowerGIT, where it is ultimately excreted in feces as microbial protein-nitrogen (NRC, 2007). There is, however, always a small amount of urea distributed in the bloodstream and other body fluids, due to the water solubility of urea. This urea finds its way into the saliva and re-enters the rumen. Urea has also been shown to pass into the rumen directly through the rumen wall from the circulating blood.

The proportion of recycled urea that is used for ruminal bacterial protein synthesis can range from 5 to 95 percent, depending on a number of factors as discussed later. In sheep fed low-quality hay, urea recycled to the GIT accounted for 80-90% of blood urea, and a higher percentage of absorbed nitrogen was retained (Bunting *et al.,* 1987). Generally, salivary transfer of urea dominates in ruminants fed forage diets, but when higher concentrate diets are fed most of the urea transfer is via the blood (Lapierre & Lobley, 2001). Houpt (1959) used an isolated rumen procedure,in anesthetized sheep, to demonstrate that urea was secreted from the bloodstream into the rumen in amounts about 15 times greater than by way of saliva. This secretion or recycling appears to occur under normal conditions. It has been proposed that this mechanism will supply nitrogen to preserve the rumen microbial population when the feed supply is limited or of very low nitrogen content and thereby improve the quality of protein supply. For sheep and goats in semi-arid and arid environments, this urea recycling enables them to meet maintenance protein requirements when forage quality is low (NRC, 2007).

Numerous experiments with beef cattle and sheep have led to the view that the quality of dietary protein is of relatively little importance because all nitrogen sources are largely converted to microbial protein in the rumen and the host animal is presented with protein of more or less standard quality regardless of its diet. Research has shown that the biological value of proteins is much less variable for ruminants than for non-ruminants (Loosli & McDonald, 1968). Johnson *et al.* (1942) found biological values of approximately 60 for lambs on a 12% protein diet, regardless of the protein source.

These claims have been challenged by more recent studies (Zeremski, 1989), in which he claims that protein feedstuffs in which the proteins pass through the reticulum-rumen to a greater extent non-degraded and reach the duodenum, result in greater weight gains in lambs if sufficient energy is available. This was confirmed in studies conducted by Ružić-Muslić (2006, 2007d) where the protein source has significantly (P <0.01) improved the ADG and final body weight of fattened lambs, with the best performance achieved by lambs in the treatment diets with fishmeal.

Similar results, in terms of the effect of protein sources on the production performance of fattened lambs, were obtained by Miller (1978), Beermann *et al*. (1986), Walz *et al*.(1998). In all of these studies, it was evident that the fish meal, as a protein source, improves growth and feed efficiency in lambs. This was explained by the fact that themicrobial protein is insufficient to meet metabolic requirements in amino acids necessary for the growth of animals, so the use of a protein source with a high content ofundegradable protein, results in superior performance.

Microbial protein contains lower levels of Methionine than high-quality food protein. Loosli & Harris (1945) observed improved lamb performance when urea was supplemented with Methionine supplements. Microbial protein has a biological value of 60 to 70. When low-quality protein sources like maize (zein) are fed, their biological value is improved by rumen microflora, while the biological value of high-quality protein sources is degraded by bacteria (Loosli & McDonald, 1968).

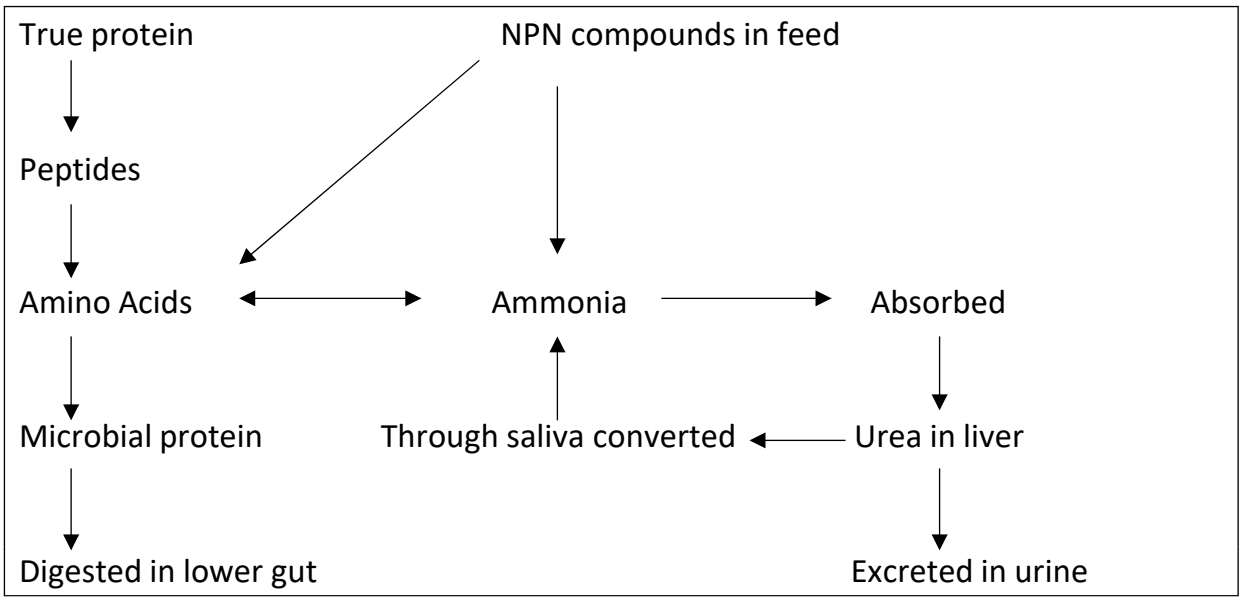

Figure 2.1: Schematic diagram of digestion and absorption of nitrogenous compounds in ruminants (Panday, 2010).

2.2.2 Dietary factors affecting urea utilization

The efficiency with which urea is being utilized by the ruminant animal depends on a number of factors as discussed below.

a) Nitrogen

The influence of exogenous nitrogen on the synthesis of microbial protein has been tested in many experiments. As discussed above, ammonia is vitally important

for the production of microbial protein and thus ammonia utilization will be greatest whenever ammonia is the first limiting factor in microbial synthesis. The level of urea needed to meet the optimal amount of ammonia in the rumen depends on (1) the amount of ammonia from degraded nitrogenous compounds contained in other components of the diet; (2) amount of recycled endogenous urea; and (3) levels of other important components (energy, minerals, etc.). In general, at low-protein intakes and when low-quality roughage diets are fed, ruminants tend to have lower blood concentration of urea, lower urinary urea excretion, and increased transfer of urea to the GIT (Robbins *et al.,* 1974). Wegner *et al*. (1940) observed that as the level of protein in a culture medium was increased, the amount of urea converted to protein markedly decreased. Balance experiments with animals fed varying levels of true protein confirmed these *in vitro* experiments (Wegner *et al*., 1941).

Urea utilization is also influenced by the quality of protein or types of AA supplied by the diet, as some may increase and others decrease the utilization (Pearson & Smith, 1943). Loosli & Harris (1945) reported an increase in nitrogen retention in lambs when methionine is added to a urea-based diet, while Burroughs *et al.* (1951) were unable to demonstrate any marketable differences between proteins of different AA compositions. Gallup *et al.* (1952) also observed no differences in the storage of nitrogen when urea supplemented cottonseed meal, soybean meal, or maize gluten meal, even though their AA composition is markedly different. Natural proteins have different solubilities or rates of hydrolysis in the rumen. The more soluble the protein, the more rapidly it is hydrolyzed in the rumen and converted to ammonia, and for this reason, it might be more competitive with urea.McDonald (1952) demonstrated that when an insoluble protein like zein is fed, thereis very little increase in the ammonia content of rumen digesta, but when casein or gelatin are fed large amounts of ammonia were liberated.

Gallup *et al.* (1952) observed the effects of increasing levels of urea supplementation on a high-concentrate diet with 7,1% CP. Urea was added to increase the protein value and obtain diets with 8,5%; 10,2%, which increased the nitrogen retention. Further supplementation of urea to obtain a 12,5% protein diet failed to further increase nitrogen retention. The digestibility of organic matter was increased from 58% in the basal diet to 71,5% or more in the 10,2% and 12,5% protein diets, and crude fiber digestibility was increased two-fold.

b) Source of energy and carbon skeletons

The single most important factor influencing the amount of urea a ruminant animal can use is the digestible energy or total digestible nutrients (TDN) content of the diet (Stanton & Whittier, 1998). Carbohydrates normally provide the main

source of energy and carbon skeletons for microbial synthesis. The main source of carbon chains for microbial synthesis is fermented carbohydrates and preformed dietary amino acids. The degraded proteins are the main source of branched-chain carbon skeletons. There is evidence that urea is utilized less efficiently when it's fed with only hay or other forages, compared with diets containingsome starch or cereal grains (Loosli & McDonald, 1968). Increased soluble carbohydrate supply can result in an increased rate of fermentation, increased propionate supply, and increased incorporation of rumen ammonia into microbial protein (Dove & Milne, 1994; Trevaskis *et al.,* 2001). McDonald (1952) reported a decrease in rumen ammonia levels when starch was added to the rumen of sheep after they had been fed a casein-containing diet, which suggests that starch provided the energy needed by bacteria to utilize ammonia.

Gallup *et al.* (1952) reported starch to be the major source of energy for urea utilization. In that study, he founded that the usefulness of urea supplementation is influenced by the type and ratio of roughage to concentrate. When a high-hull basal diet, low in digestible nitrogen, and available energy are supplemented with urea, it failed to significantly change the negative nitrogen balance. Nitrogen output in urine exceeded the amount of nitrogen absorbed, and thus only a small amount of supplemental nitrogen was utilized. A medium-hull diet did perform better, but in general,the composition of the high and medium-hull diets was unfavorable for efficient utilization of urea. The low-hull diet which contained increased amounts of maize resulted in a significant increase in nitrogen retained and a positive nitrogen balance of 1,22 grams compared with 0,26 grams on the medium-hull diet.

Pigden (1971) reported that the lignocellulose complex accounts for most of the energy in mature forages. He related the total dietary nitrogen levels to the total digestible energy of forages and suggested that total dietary nitrogen content of 1% is sufficient for the utilization of forages with less than 50% digestible energy. However, for forages with more than 50% digestible energy, a total of 1,5% dietary nitrogen is needed and a total of 2% when there is 20% of starch in the diet. However, several investigators have reported no improvement in digestion of forages when supplemental nitrogen is provided (Nelson & Waller, 1962; Donefer *et al.,* 1969; Williams *et al.,* 1969). Campling *et al.* (1962) also reported an increase in intake and digestibility of oat straw in cows when urea was infused into the rumen.

Two attempts have been made to predict by mathematical formulae (Satter & Roffler, 1973; Burroughs *et al*., 1974a) the usefulness of urea supplementation based upon the total protein present in the diet as an index rumen protein breakdown and in relation to the quantity of TDN in the diet as an index of fermentable energy. These attempts predicted that on high concentrate diets with TDN values higher than 75% on a DM basis, some ammonia will be synthesized into additional microbial protein at protein levels below 12 or 13%. At higher protein levels, the formulae

predict thatprotein breakdown will be sufficient to maximize microbial growth, and ammonia from added NPN will not increase microbial synthesis and therefore will be utilized less efficiently.

Clark & Quin (1951) demonstrated that the digestibility of dry matter and cellulose was not changed by the addition of urea-molasses, but the feed intake did increase due to an increase in digestion rate. Coombe & Tribe (1962) also found that the digestibility of straw in sheep was unchanged by the addition of urea, but the rate ofdigestion was increased.

c) Effect of other dietary factors

When urea substitutes natural protein it changes the quantity and quality of the minerals available for the rumen microbes and the host animal, but the requirements of the rumen microbes and host animal do not change with the presence of urea. Sulfur has long been recognized as an important element for rumen microorganisms and its metabolism closely related to nitrogen metabolism. Supplementation of sulfur to a sulfur deficient diet improves ruminant performance by enhancing bacterial protein synthesis in the rumen and improving AAbalance (Morrison *et al.,* 1990).

Availability of minerals may be altered by substitution, for example, natural sulfur may be more available than the added sulfur. Because major diet changesare often made when urea is included, other dietary ingredients will dictate which mineral elements need to be supplemented when urea replaces intact protein (Ovejero & Hogue, 1970). When lambs were fed a purified diet with urea as the only nitrogen source and no added sulfur, lambs lost body weight and were in a negative balance for both nitrogen and sulfur. When the same diets were supplemented with sulfates it resulted in a positive nitrogen balance and weight gain (Thomas *et al.,* 1951). Sulfur is needed by rumen bacteria for the synthesis of methionine and cystine as well as thiamine and biotin, thus such changes would be expected when sulfur is added to the diet.

Moir (1970) reported a dietary requirement of sulfur by sheep as nitrogen: sulfur ratio of 10:1. Hatfield (1972) showed that a ratio of 15:1 was superior to a 10:1 ratio for the fatting of cattle, presumably due to species differences in the relative production of keratin. Gutierrez *et al.* (1996) found that the nitrogen: sulfur ratio of rumen bacteria ranged from 8:1 to 31:1 (mean of 21.6:1) and concluded that a ratio of 20:1 will be adequate to supply rumen microbial requirements. Total sulfur content is therefore a function of total nitrogen content.

High levels of true antibiotics should have some effect on the rumen microbial population, but at low concentrations, it may have no significant effect. Prescott (1953) reported that many antibiotics are nonspecific inhibitors of urease in rumen fluid. It would be expected that a decrease in the rate of urea hydrolysis as a result of antibiotics will improve urea utilization. Brown *et al.* (1960) conducted an experiment on 42-day old calves receiving four different starter diets that differed in protein level. After 3 weeks the calves that received the antibiotic-fortified diet had better weight gains, but there was no increase in feed intake. Calves receiving antibiotics had satisfactory growth on a 3% lower protein diet than required for similar gains without antibiotics. Beneficial effects had been reported for the use of chlortetracycline in fattening lambs receiving urea supplemented diets (Cahill & McAleese, 1964).

The ruminal hydrolysis of urea by urease usually occurs at a faster rate than the microbial utilization of the produced ammonia, and therefore it's used rather inefficiently. The greatest efficiency of urea utilization occurs when there is a synchronized supply of ammonia from urea and carbon skeletons from other rapidly fermentable dietary constituents. Treatment of carbohydrates to increase the rate of hydrolysis and reduction or inhibition of urease activity within the rumen fluids are two ways to increase the efficiency of urea utilization. It had been reported that high inclusion levels of urea will inhibit urease activity (Caffrey *et al.,* 1967a; Chalupa *et al.,* 1970), although most conditions will allow for the complete hydrolysis of urea.

Ludden *et al.* (2000) investigated the effect of the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) on ruminal protein metabolism and fermentation. They supplemented a diet containing 2% urea with 0.125 to 4.0 g/day of NBPT and identified linear dose effects on decreases in urease activity, ruminal ammonia concentration, and nitrogen retention, but ruminal urea concentration and urinary nitrogen excretion increased linearly. Urease activity and ureolytic microbes inside the rumen are crucial in the effective utilization of urea in the rumen.

2.3 Potential constraints and limitations

The reasons for preferring urea over other sources of rumen degradable protein (RDP) are that urea N is cheaper on an N basis than any other feedstuff and transportation and storage are cheaper and more convenient due to the concentrated nature of urea (McPherson & Witt, 1968). Urea, however, is used less efficiently compared with other sources containing natural protein (Broderick *et al.,* 2009). The reason for this is due to the rapid hydrolysis of urea which is producing ammonia more rapidly than rumen bacteria are capable of utilizing, and this resultsin accumulation and absorption of rumen ammonia and subsequent excretion of urea in the urine (Golombeski *et al*., 2006; Highstreet *et al*., 2010). Therefore, utilizing urea as a source of RDP has potential difficulties for ruminant nutritionists.

One strategy of nutritionists to improve urea utilization is to more closely synchronize the fermentation of energy and the availability of ammonia in the rumen (Johnson, 1976), by either increasing rate of carbohydrate fermentation or decreasing the degradation rate of urea.

Reid (1953) already recognized the potential limitations of urea in an extensive review of the use of urea in cattle diets. He recognized that much of the urea N is lost in urine due to the rapid rate of urea hydrolysis. The author also observed that urea was utilized less efficiently when the diet already had a crude protein content of12% or more and that urea inclusion above 1% of diet DM resulted in reduced feed intake due to palatability problems. Almost all reports on decreased animal production performance when urea is fed are attributable to a reduction in feed intakedue to palatability (Polan *et al*., 1976; Kertz, 2010). Several authors have challenged these recommendations of Reid. Kwan *et al.* (1977) found that 1% urea was used rather efficiently by dairy cows up to a CP content of 16.6% but supported the recommendation of Reid (1953) that inclusion levels of urea above 1% may result in reduced performance due to reduced feed intake. Broderick *et al.* (1993) did not observe reduced feed intake when replacing natural protein with 1.33% urea but did observe reduced feed intake with 1.63% urea inclusion.

Burroughs *et al.* (1975) suggested that urea utilization is probably more a function of rumen degradable protein supplied by the diet and the availability of rapidly fermentable energy to capture ammonia in the rumen rather than the absolute CP content of the diet. Kertz (2010) suggested that urea can be included up to 1,5% under certain circumstances without a depression in feed intake. When urea is fed in a total mixed ration (TMR) it is less likely to depress feed intake as opposed to discreet feeding, which is probably due to the amount of readily available carbohydrates in the rumen (Kertz, 2010). Urea is less likely to cause a decrease in feed intake or cause toxicity when it's fed alongside a readily available source of fermentable energy (Bartley *et al.,* 1976). Additionally, increasing levels of fermentable energy in the diet will reduce the rumen pH which will decrease absorption of ammonia through the rumen wall (Bartley *et al.,* 1976). This supports the theories of Burroughs *et al.* (1975) that urea fermentation potential is positively related to the amount of total digestible nutrients (TDN) in the diet.

Another potential disadvantage of using high inclusion levels of urea in the diet of ruminants is urea (ammonia) toxicity. Ammonia toxicity is seen most of the time in animals newly introduced to diets containing NPN as their predominant protein source. Ammonia is a weak base with a pKa of 8.8 at 40°C, therefore, the ratio of ammonia to ammonium ions has a close relationship with ruminal fluid pH. When urea enters the rumen it's broken down to ammonia by the action of the enzyme urease. If excessive ammonia is absorbed through the permeable lipid layer of the rumen mucosa into the bloodstream it can overwhelm the detoxifying capacity of the liver and therefore unable to convert it back to urea, which results in ammonia

toxicity and simultaneously there is a rise in rumen pH to such an extent that the rumen ceases and fails to function normally. The alkaline buffering capacity of the rumenfluid is also less than the acid buffering capacity.

Bartley *et al.* (1976) presented data that suggested that ammonia toxicity is poorly correlated to ammonia concentration in the rumen. They demonstrated that ammonia toxicity was more closely related to rumen fluid pH. Rapid hydrolysis of urea results in a build-up of ammonia, which results in a sharp increase in rumen pH as a result of the ionization of ammonia molecules which removes free hydrogen from the solution. The effect of pH causing ammonia toxicity was confirmed by Kertz *etal.* (1983) in a study in which they added ammonia equivalent amounts of ammonium chloride (already ionic) and urea, which requires the addition of hydrogen ion to ionize it. The addition of ammonium chloride resulted in an increased concentration of ammonia in the rumen, but no increase in rumen pH and subsequently no ammonia toxicity.

Animals are often found dead as the progression of ammonia toxicity is very rapid. Acute ammonia toxicity symptoms in ruminants appear progressive as described in NRC (1976): The animal becomes nervous and uneasy, salivates excessively, and demonstrate muscular tremors, these symptoms are followed by incoordination, respiratory difficulty, and frequent urination and defecation, the front legs begin to stiffen, and the animal becomes prostate; violent struggling, bellowing, and terminal tetanic spasms are found in most animals, the jugular pulse is marked, and bloating is common; death occurs within 0.5 – 2.5 hours after the first signs were observed.

The most common causes of toxicity from urea supplementation include: improper mixing which results in concentrated pockets; calculation error resulting in excessive supplementation; animals are not properly adapted over a period of time; inclusion of urea into a diet containing predominantly poor-quality roughage; feeding urea with other diet components that raise the rumen pH because urease activity is increased in an alkaline environment. Several factors make animals more prone to toxicosis, which includes: feeding urea-containing diet to fasted animals; inadequate water intake; elevated body temperature increases the activity of urease; liver disease; stressed animals (Whitehair, 1989).

Due to the rapid progression of toxicosis caused by urea, treatment is often not possible. Several modes of treatment have been suggested in the literature. In the case of toxicity, vinegar is an effective solution. Mix equal amounts of vinegar and water and dose half a bottle per calf/sheep and 2-4 bottles for cattle (1 bottle = 750ml). This will neutralize the toxic effect by improving the blood acid-base status and retard bacterial production of free ammonia. This will lower the pH of the rumen

and as reported by Coombe *et al.* (1960) and Hogan (1961), at a high pH, ammonia is absorbed at a much faster rate. Acetic acid can also be used instead of vinegar. Oral charcoal may also help decrease ammonia absorption.

2.4 Guidelines when using urea as a feed component in ruminant diets

2.4.1 Adaptation

When urea is introduced into the diet the performance is usually less than those animals fed only natural protein. The lower performance of fattening cattle fed urea as a source of nitrogen may be due to the lower performance during the first part (Meiske & Goodrich, 1966). It takes about 10 days for the rumen microbes to adjust to any drastic changes in the diet. Cattle require at least five to seven days of small increases in concentration. This adaptation period needs to be repeated even if urea or other NPN is removed for a very short time. A long-term trial with fistulated ruminants found that nitrogen balance, as a percentage of nitrogen uptake to absorbed nitrogen, decreased until the fourth month and then increased again (El-Shazly *et al.,* 1981).

Retention of nitrogen by ruminants when urea is fed tends to increase with the length of the feeding period until a plateau is reached. This period of increased efficiencyof utilization is sometimes referred to as the “adaptation period”. Maximum utilization of urea by lambs fed a semi-purified diet occurred after 35 days (Welch *et al.,* 1957). Smith *et al.* (1960) observed a 2% increase in retention of absorbed nitrogen in lambs with each consecutive 10-day feeding period up to 50 days, with no measurable change in OM digestibility. Several other authors have observed this response (Repp *et al.,* 1955a; Anderson *et al.,* 1959; McLaren *et al.,* 1959; Campbell *et al.,* 1963), while other authors did not observe the same trend (Miller & Morrison,1942; Ewan *et al.,* 1958; Schaadt *et al.,* 1966). No evidence of adaptation to urea wasobserved in cattle that were fed natural diets high in crude protein (Johnson *et al.* 1967; Oltjen *et al.,* 1969). Caffrey (1965) presented experimental evidence which suggested that the response observed with time is an adjustment to the nutritional regimen rather than an adjustment to urea. Even with the infusion of urea intravenously for 60 days, he observed no changes in the concentration of ammonia in blood or rumen.

2.4.2 Level and frequency of feeding

Prior discussions have enumerated the effects of dietary protein levels. The effects of frequency-of-feeding are sometimes confounded with changes in total diet intake, but most experimental data provide evidence that a constant or continuous intake of urea results in better utilization compared with abrupt or periodic intake. Aftera single administration of dietary urea, the ammonia concentration in the rumen rapidly increases, peaking at 60-90 minutes and then decline to normal levels

4-5 hours after initial administration (NRC 1976). Rumen microbes strive in a steady environment, therefore, providing urea more frequently at lower levels may improve the efficiency ofutilization by decreasing rumen ammonia levels.

Several investigators have reported improved animal performance with increased feeding frequency. Bohnert *et al.* (2002b) observed a tendency for forage and total DM and OM intake to be greater, when urea was supplemented once a day compared with once every third day, or once every sixth day. They reported that as thefeeding frequency decreased from daily to every sixth day, lambs receiving a high and low-DIP supplement had an 8% and 19% decrease in forage and a 7% and 17% decrease in total DMI, respectively. They suggested that infrequent supplementation may have impaired rumen function for a period of time because of the larger quantity of supplement provided during a supplementation event as the feeding frequency decreased. Similar results have been obtained by Currier *et al.* (2004b) when they compared daily versus every second-day urea supplementation. Prior (1974) found no difference in performance when lambs were fed a soybean meal diet twice or 12 times daily. However, he found that feeding urea supplemented diets twice daily produced negative nitrogen balances compared with positive balanceswhen fed 12 times daily. Other authors failed to find any significant effect of feeding frequency on total DMI in ruminants consuming low-quality forage (Krehbiel *et al*., 1998; Huston *et al*., 1999a).

2.4.3 Liquid supplements

These products are primarily molasses-based materials with urea or other NPN compounds as the major nitrogen source. Many diets with a high content of low-quality roughage are very low in fermentable energy, protein, and some macro- and micro-minerals. To optimize the rumen environment in terms of the availability of readily fermentable carbohydrates, ammonia N, and minerals, supplementation of urea and molasses in the form of blocks or as liquid feed is often suggested (Preston & Leng, 1987). The primary purposes of feeding liquid molasses supplements are to supply energy and protein substitutes, to prevent unfavorable loss of weight, and to serve as a carrier for feed ingredients such as vitamins and minerals. The use of such liquid supplements has several advantages as listed below (NRC, 1976):

1. Supply readily available energy for rumen microbes to convert urea or other NPN into microbial protein.

2. Serves as a transport system for many soluble micronutrients and other non-nutritive dietary additives in properly formulated supplements.

3. Serves as a binding agent which reduces dust hazards and feeds losses due towind erosion.

4. Provides a cohesive medium for combining the supplement with other ingredients of the diet that will improve uniformity, especially with high-forage diets.

5. Improves palatability of diets high in low-quality forages, which ultimately improves production by increasing feed intake.

6. Fits well with certain mechanical feeding systems.

There are some reported problems associated with liquid supplements:

1. Needs to be kept in solution or suspension over a period of time with different environmental temperatures.

2. Requires special equipment for convenient addition and proper mixing into the rest of the diet.

3. Possibly result in overconsumption and large variation in individual intakes when self-fed.

4. Causes some corrosive effect on the equipment.

3. A brief overview of some plant natural protein sources in ruminant nutrition

3.1 Cottonseed oilcake meal

Cottonseed meal is the by-product of oil extraction from cotton seeds. As a protein-rich feed, cottonseed meal is a common source of protein for ruminants, especially in cotton-producing areas. It can replace 100% of soybean oilcake meal in ruminant diets when the economics thereof is to be considered. Several methods are used to extract cottonseed oil, resulting in different types of cottonseed meal (Figure 2.2). This situation is slightly different from that of other major oilseeds such as soybean and sunflower, where one process is usually dominant. As a result, there is a wide range of cottonseed meals differing in their protein, fiber, and oil content.

The protein content ranges from 30% DM for non-dehulled cottonseed meals upto 50% DM for fully dehulled meals. The crude fiber content varies from 25% (non- dehulled) to 5% (fully dehulled). The different methods used for oil extraction also explain the large range of residual oil present in cottonseed meals. Solvent extracted meals can contain less than 2% oil, similar to other major oilseed meals, but many cottonseed meals contain oil values in the range of 5-10%. The protein of cottonseed oilcake meal is less rich in lysine than soybean oilcake meal (4% *vs* 6% of the protein) (Nagalakshmi, 2007).

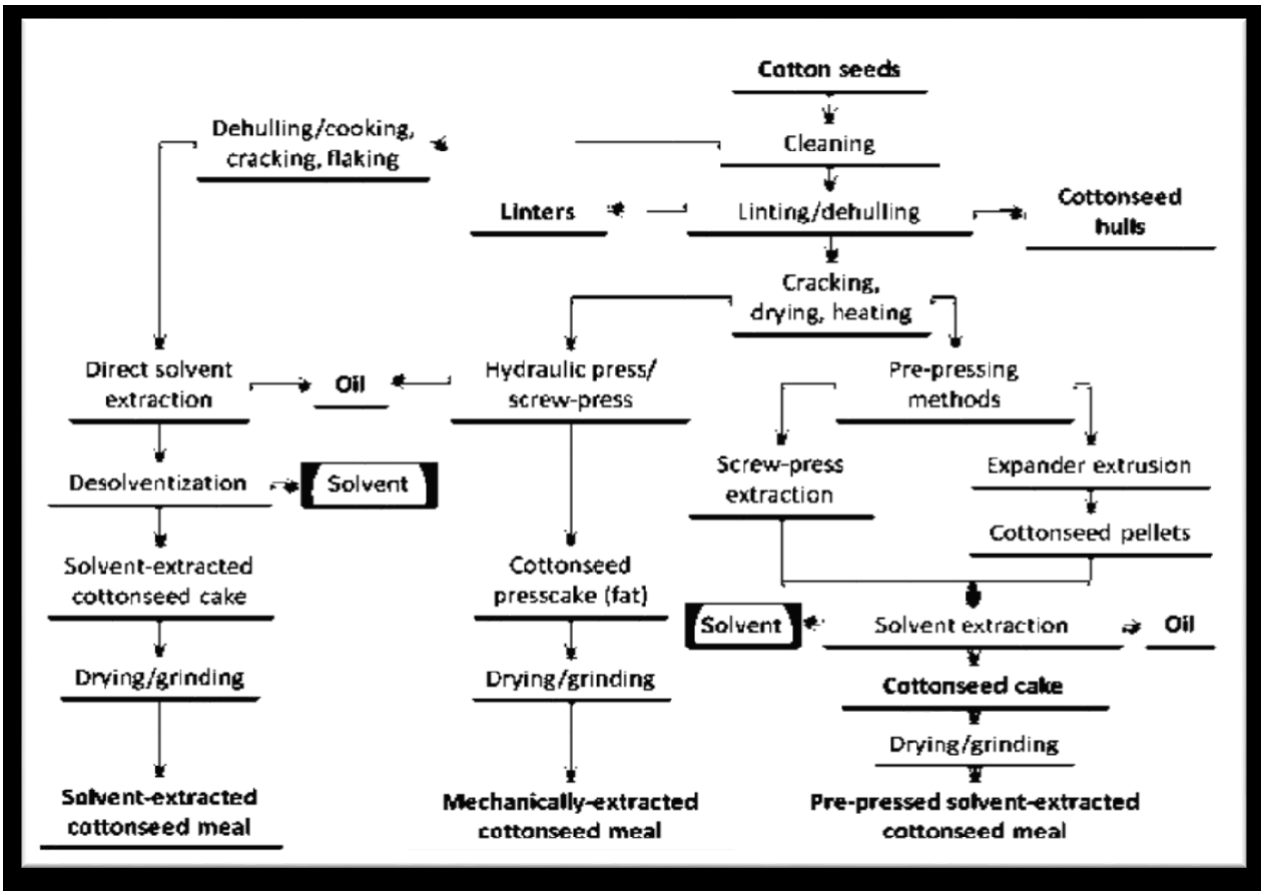

Figure 2.2: Schematic diagram of the different methods used to extract cottonseed oil (Heuze *et al.,* 2013).

- **Mechanical extraction**: This method of cottonseed oil extraction usually uses a hydraulic press or screw press (expeller). The cottonseeds may be dehulled, cracked, dried, or heated before being fed to the press. The cake is then dried, ground, and then processed into large pellets (Ash, 1992). This method of extraction is not very efficient and up to 20% of the seed oil may remain in the pressed cake, depending on the technology used (O'Brien *et al.,* 2005).

- **Direct solvent extraction process:** as in mechanical extraction, the seeds may be dehulled, cooked, cracked, and flaked, but the oil is extracted by solvent (usually hexane) alone. The extracted cake is heated to eliminate the solvent and then generally ground into a meal (Ash, 1992).

- **Pre-press solvent extraction:** This method combines a mechanical extraction (screw press or expander) step, which reduces the oil by one-half to two-thirdsof its original level, and solvent extraction, which results in a 97% oil extraction rate. The dehulled, cracked, dried, heated or flaked cotton seeds are first screw pressed or expanded and the pressed flakes or pellets are then solvents extracted.

Cottonseed oilcake meal is a good protein source for ruminants, it's palatable with a nutritive value (for dehulled meals) slightly lower (85-90%) than that of soybean oilcake meal and it is among the least expensive sources of protein in some regions (McGregor, 2000). Brand & van der Merwe (1993) conducted a trial where feedlot lambs received diets with different protein sources ((urea, urea plus fish-meal, urea plus cottonseed oilcake, urea plus bitter lupins, or bitter lupins only). In that trial, they did not observe differences in ADG, FCR, or days in the feedlot. They attributed the lack of response to the different protein sources to the fact that the undegradable protein (UDP) requirement of lambs over 25 kg was fulfilled by the triticale/oat grain mixture.

Kandylis *et al.* (1999) observed no difference in fattening lambs fed diets where cottonseed oilcake meal substituted sunflower oilcake meal at different proportions. They concluded that CSOCM was accepted readily by growing lambs and can be included as 100% of the supplemental protein in diets of fattening lambs. Khan *et al.* (1997) observed a slightly lower ADG for lambs receiving a TMR with CSOCM (213g/day) compared with lambs receiving soybean oilcake meal and rapeseed meal (233-244g/day). Ward *et al.* (2008) also observed lower ADG (170 vs 200g/day) and diet digestibility (65% vs 75%) when soybean oilcake meal was substituted with CSOCM in a high concentrate diet. In diets for pre- and post-weaning calves, CSOCM gave the same weight gains as rapeseed meal or soybean oilcake meal (Coppock *et al*., 1987) or slightly lower gains than soybean meal (Yazdani, 2005).

3.2 Soybean oilcake meal

A highly palatable feedstuff, soybean meal is characterized by high protein content (from 43 to 53%) and low crude fiber content (less than 3% for the dehulled soybean meals). It has a very good amino acid balance and contains high amounts of lysine, tryptophane, threonine, and isoleucine, which are often lacking in cereal grains (McDonald *et al.,* 2002). Soybean meal is an important part of the diets of ruminants due to its high amount of rumen-degradable protein (more than 60%), good amino acid balance, and high cell-wall digestibility (INRA, 1988). It is also very palatable to ruminants. Inclusion levels in ruminant and pre-ruminant diets are about 35% in dairy cows and beef, 30% in ewes, and 20% in calves and lambs (Ewing, 1997).

While soybean meal is well degraded in the rumen and provides ammonia, amino acids, and peptides for rumen microbial protein synthesis, it may not provideenough undegraded protein to meet the demands of highly productive animals.
Therefore, an important line of research has consisted in developing techniques aiming at improving the rumen by-pass quality of the soybean meal protein. Many methods have been tested over the years:

Mechanical and thermal processing: extruding, extruding-expelling, heating, various combinations of heat and pressure, etc.

Chemical treatments: alcohol, formaldehyde, NaOH, NaCl, xylose, tannins, heated Ca salts, bentonite, acids, alkalis, encapsulation with blood, zein, or fat (Wacyk*et al*., 2000; Colmenero *et al*., 2006; Castro *et al*., 2008).

Replacing part of the soybean meal with non-protein sources of nitrogen such as urea or other protein sources such as (cottonseed meal, sunflower meal, etc.) has also been extensively studied. In a study conducted by Pires *et al.* (2004) where there was a total replacement of soybean meal by urea in high grain diets for beef cattle. The diets contained 13% CP and were composed of 80% concentrate and 20% raw bagasse. The cattle receiving the urea diet had a higher DMI, higher ADG, and a betterFCR compared with the cattle receiving the soybean meal diets. In the rumen fermentation study, they observed a higher rumen ammonia-N concentration in cattle receiving the urea diets.

Paengkoum & Bunnakit (2009) conducted a study in which they replaced soybean meal with cassava pulp mixed with urea gelatinizes (Caspurea) in concentrate diets of beef cattle. Soybean meal was replaced at levels of 25, 50, and 75%, and diets were on an iso-nitrogenous basis. DM digestibility was lowest in the 75% replacement diets, and decreased linearly (P <0.01) and quadratically (P <0.01) as the level of Caspurea increased. Rumen ammonia-N was highest at 75% replacement and increased linearly (P <0.01) and quadratically (P <0.01).

Claypool *et al.* (1985) supplemented dairy calves with canola meal, cottonseed meal, or soybean meal as protein supplements. The trial consisted of an eight-week pre-weaning period where calves were offered a fresh starter diet, free choice, and aneight-week post-weaning period where calves were fed 2.27 kg per head daily. The pre-weaning ADG for calves fed canola, cottonseed, and soybean meals were 0.58,
0.62 and 0.62 kg and for the post-weaning period were 0.89, 0.89 and 0.92 kg. There were no significant differences.

As with cattle, there have been numerous attempts at replacing soybean meal in sheep diets with locally available and less expensive protein sources. In an experiment conducted by Irshaid *et al.* (2003), they evaluated sunflower seed meal as a substitute for soybean meal in diets of fattening Awassi lambs. The sunflower seed meal replaced the soybean meal at 50% and 100%. In the digestibility study, there were no significant differences observed between lambs fed the experimental diets in digestibilities of DM, OM, CP, CF, NDF, ADF, or N balance. In the growth study, therewere no significant differences in the average final body weight, ADG, and FCR amongthe treatments. These experiments showed that sunflower seed meal can replace soybean meal as a protein source in diets of fattening lambs.

3.3 Sunflower seed meal

Sunflower meal is one of the major protein meals used for livestock feeding, particularly for ruminants. It is generally a valuable and safe product, whose protein, fiber, and oil contents are highly variable and driven by variations of the oil extractionprocess (Hesley, 1994). Its protein content ranges from 23% DM for some non- dehulled, mechanically-extracted meals, to more than 40% for highly decorticated, solvent-extracted meals. However, the usual ranges for protein are 29-33% DM for non- dehulled meals and 35-39% DM for dehulled and partially dehulled meals. The fiber content is directly linked to the presence of hulls: crude fiber ranges from 27 to 31% DM for non-dehulled meals and from 20 to 26% for dehulled and partially dehulled sunflower meals. The lignin content is important, in the 9-12% range, even for dehulled meals. Solvent-extracted sunflower meals contain about 2-3% DM of residual oil, but mechanically-extracted meals may contain up to 30% oil depending on the amount of pressing. This oil content gives expeller meals higher gross energy(22 MJ/kg DM or more vs. 19 MJ/kg DM for solvent-extracted meals), but these meals contain less protein than solvent-extracted ones (Lusas, 1991).

Richardson *et al.* (1981) conducted a study in which the nutritional value of sunflower meals as a protein supplement for growing cattle and sheep was determined by the evaluation of treatment effects on *in vivo* digestibility, N retention, ADG, FCR, and wool growth. In the lamb feedlot experiment, sunflower meal replaced CSOCM in a sorghum-based diet. In the 12% CP diet, it had no effect onADG or FCR. The same was reported for the 8% CP diet, except for the higher wool growth observed for the lambs receiving the sunflower meal diets (6.95 vs 5.90 g/cm^2). They concluded that sunflower meal promoted better wool growth than cottonseed meal due to its higher content in sulfur-containing amino acids.

Richardson *et al*. (1981) substituted sunflower meal for cottonseed meal in growing and finishing diets for steers at 0, 5.5, 11, and 22% of diet DM. They reportedequal total diet digestion for steer calves fed cottonseed meal and sunflower meal when fed at iso-nitrogenous and equal fiber levels up to 11 percent sunflower meal. Digestibility of dietary dry matter and organic matter was highest (P <0.05) for the 22% sunflower meal treatment. The same authors also reported equal digestibility ofhigh-forage diets for steer calves when sunflower meal was substituted for urea as a nitrogen source and fed at 0, 5, 10, and 20% of diet DM.

Jordan *et al.* (1998) compared sunflower meal with soybean meal and a sunflower-soybean meal mixture in iso-nitrogenous supplements in maize-based finishing diets that also contained 1% urea. The urea and sunflower meal provided adequate ruminal-degradable nitrogen, with the undegradable nitrogen provide by

the maize (Milton *et al*., 1997). No differences were detected for ADG, FCR, or carcasstraits due to treatment.

3.4 Brewers grains

Brewer's grains are used to feed ruminant and monogastric animals. They are palatable and readily consumed when in good condition. Brewer grains are quite rich in protein (27-33% DM), which makes them a valuable source of protein. The protein value can be affected by the heat applied during the brewing process, which can be beneficial to ruminants but tend to be detrimental for monogastric animals. Brewer grains are also relatively rich in fiber (ADF 17-26% DM), which makes them suitable for ruminants fed concentrate-rich diets, but less so for pigs and poultry.

Wet brewer's grains are bulky feed with low energy content, which can limit their use. Brewer grains have a good protein value for ruminants and are relatively rich in rumen undegradable protein compared with feeds derived from other plants. Brewer grains are thus often used in ruminant production systems with high requirements for by-pass protein, such as high-yielding dairy cows. Pereira *et al.* (1998) reported the amount of by-pass protein doubled when the drying temperature rose from 50^{o}C to 135^{o}C. The effective nitrogen degradability of brewer'sgrains reported in feed tables and the scientific literature is about 41-49% (Batajoo& Shaver, 1998; Volden, 2011).

Brewer grains have been included at rates up to 40% in diets of growing cattle (Ewing, 1997). Geron *et al.* (2008) conducted a study in which cattle were fed diets containing 0, 8, 16, and 24% of brewer's grains. In that study, there was no effect of levels of brewer's grains in diets on rumen pH and rumen NH_3-N concentrations. Theyconcluded that the inclusion of brewers grains of levels up to 24% of diet DM did notalter the processes of rumen fermentation and digestion kinetics.

Aguilera-Soto *et al*. (2007) conducted two trials in which lambs received diets containing incremental levels of wet brewers' grains (0, 150, 300, 450, 600 g/kg DM). In the feedlot trial, they did not observe significant differences in DMI, ADG, and FCR between lambs fed the different diets. In the cannulated animals they did not observe differences in the digestibility, however, they did observe increased concentrations of NH_3-N as the levels of wet brewer's grains increased.

3.5 Gluten 20 and 60

Both of these two animal feeds are by-products of the manufacture of starch from maize grain (*Zea mays* L.) by wet milling (Hoffman & Baker, 2010). It consists mostly of gluten obtained during the separation of starch. Maize gluten meal is a protein-rich feed (about 60%) and is a different product from maize gluten feed (about 20% protein). Maize gluten meal is the plant protein feed that provides the most rumen undegradable protein, ranging from 45 to 50% DM (NRC, 2001; Sauvant *et al.,* 2004; Volden, 2011).

Maize gluten feed consists mainly of maize bran and maize steep liquor (liquid separated after steeping) but may also contain distillers solubles, germ meal, cracked maize screenings, as well as minor quantities of end-products from other microbial fermentations (Stock *et al.,* 1999). The nutrient composition of maize gluten feed is largely dependent on the milling process and the resultant proportions of bran, steep liquor, and other components. The proportion of steep liquor in the blend has a positive correlation with the energy and protein content (Stock *et al.,* 1999). In Table
2.1 below is a summary of the nutritional differences between the two by-products.

Table 2.1 The differences in the nutrient composition of gluten 20 and gluten 60.

Nutrients	Gluten 20	Gluten 60
Dry matter (g/kg)	88.3	90.0
Crude protein (%DM)	21.7	67.2
Gross energy (MJ/kg DM)	18.8	23.1
[1]Metabolisable energy (MJ/kg DM)	12.2	16.6
Starch (%DM)	21.5	17.6
Neutral detergent fibre (%DM)	39.6	4.1
Ether extract (%DM)	3.4	2.9
Ash (%DM)	6.9	2.1
Calcium (%DM)	1.6	0.3
Phosphorus (%DM)	10.2	4.0

Adapted from Feedipedia- Animal Feed Resource Information System, 2015 and 2018.
[1]ME (MJ/kg DM) for ruminants

Collins & Pritchard (1992) conducted a study on alternate day supplementation of maize stalk diets with soybean meal or maize gluten meal fed to ruminants. In the steer feedlot trial, they observed higher (P <0.05) ADG and FCR for diets supplemented with gluten 60 compared with soybean meal supplemented diets. In the

rumen fermentation study, they observed higher rumen NH_3-N concentrations over time when diets contained soybean meal.

Milis *et al.* (2005) studied the effects of main protein, non-forage fiber, and forage source on digestibility, N balance, and energy value of sheep rations. The mainprotein source (soybean meal vs gluten 60) did not affect nutrient digestibility, energy value, and N balance of the diets, except for an increase in crude fiber digestibilityof diets containing soybean meal. From those results, they concluded that an increasein rumen undegradable protein content does not negatively affect digestibility or nutritive value of diets if adequate fermentable metabolizable energy (FME) is provided.

Bowman & Paterson (1988) evaluated maize gluten feed in high-energy diets for sheep and cattle. Different forms of maize gluten feed (wet, dry, or ensiled) wereincluded at up to 50% of the diet in high-concentrate lamb diets and compared favorably with diets based on maize-urea or maize-soybean meal.

From the literature review, it is clear that most of the research on urea was conducted more than 40 years ago and very little has been published on urea supplementation together with different combinations of other natural protein sources, specifically CSOCM, in lamb feedlot diets. In addition, there is a perception in the feedlot industry that protein quality from different protein sources is not important when formulating diets for feedlot cattle or lambs (2018, U. Muller, Pers. Comm., Voermol). In our study, we included CSOCM which can be regarded as a high-quality source of natural protein. The purpose of this study, therefore, is primarily toinvestigate the effect of level of urea supplementation on lamb feedlot performanceand secondary to investigate the importance of protein quality when formulating diets for feedlot lambs.

Chapter 3

Effect of level of urea supplementation on growth performance and carcass characteristics of feedlot lambs

1. Introduction

The trial consisted of two experiments conducted at the small stock section on the Hatfield Experimental Farm of the University of Pretoria; South Street, Hatfield, Pretoria. The first experiment was a feedlot trial in which lambs received diets with different inclusion levels of urea. This research project aimed to determine the effect of protein quality and different inclusion levels of urea on growth performance and carcass characteristics of feedlot lambs. The second experiment (Chapter 4) was a rumen fermentation study where the effect of treatment on different rumen parameters was investigated.

The Animals Ethics Committee of the University of Pretoria approved experimental protocol and trial, with ethics approval project number EC082-17.

2. Materials and method

2.1 Experimental design

The experiment was designed as a randomized complete block design using 250 Merino lambs. The 250 lambs were blocked into five homogenous groups according to their recorded weights and sex, which allows more precise comparisons among treatments. Within each block, which consisted of 50 animals, the lambs were divided into five pens of 10 lambs each. Pens within each block had similar weights. Each pen within a block was assigned randomly to one of five treatments to ensure that each treatment was represented occurred within each block. This was done by assigning a different permutation randomly to each block (Kuehl, 2000). This resulted in a completely randomized block design, which consisted of 25 pens, therefore five pens (replications) per treatment. By using five replications per treatment, it made provision for variation in feed intake, feed efficiency, and growth performance of animals within a treatment, thus improving the accuracy of the experiment. Pens were treated as the experimental units (Kuehl, 2000). Figure 3.1 shows the random assignment of the five treatments to the pens within a block. The average body weight (kg) of each pen which was used to block lambs into homogenous blocks according to weight is displayed in Figure 3.1.

Block 1	**Pen 1**	**Pen 2**	**Pen 3**	**Pen 4**	**Pen 5**
	Treatment 1 29.12 kg	**Treatment 2 29.13 kg**	**Treatment 3 29.23 kg**	**Treatment 4 29.20 kg**	**Treatment 5 29.22 kg**
Block 2	**Pen 6**	**Pen 7**	**Pen 8**	**Pen 9**	**Pen 10**
	Treatment 5 29.17 kg	**Treatment 4 29.14 kg**	**Treatment 3 29.27 kg**	**Treatment 2 29.17 kg**	**Treatment 1 29.03 kg**
Block 3	**Pen 11**	**Pen 12**	**Pen 13**	**Pen 14**	**Pen 15**
	Treatment 3 29.22 kg	**Treatment 4 29.21 kg**	**Treatment 5 29.38 kg**	**Treatment 1 29.10 kg**	**Treatment 2 29.16 kg**
Block 4	**Pen 16**	**Pen 17**	**Pen 18**	**Pen 19**	**Pen 20**
	Treatment 2 29.19 kg	**Treatment 1 29.12 kg**	**Treatment 5 29.19 kg**	**Treatment 4 29.23 kg**	**Treatment 3 29.18 kg**
Block 5	**Pen 21**	**Pen 22**	**Pen 23**	**Pen 24**	**Pen 25**
	Treatment 4 29.19 kg	**Treatment 5 29.25 kg**	**Treatment 1 29.13 kg**	**Treatment 2 29.20 kg**	**Treatment 3 29.12 kg**

Figure 3.1 Randomised complete block design used in the feedlot trial.

2.2 Experimental diets and treatments

The trial consisted of five experimental diets with different inclusion levels of urea. The control diet (treatment 1) contained 0% urea. Inclusion levels of urea on an as is basis were 0% in treatment 1; 0.42% in treatment 2; 0.83% in treatment 3; 1.25% in treatment 4 and 1.66% in treatment 5. As urea inclusion increased, the inclusion levels of cotton seed oilcake meal (CSOCM) in experimental diets decreased, with inclusion levels ranging from 15.42% (treatment 1) to 0.69% (treatment 5). These experimental diets were used to determine what the optimum inclusion levels of urea are.

The experimental diets were a total mixed ration (TMR) in a pelleted form that was formulated as a feedlot lamb diet according to the National Research Council (NRC, 2007). The diets were formulated on an iso-energetic, iso-nitrogenous, and iso-fibrous basis so that differences in results were not due to differences in metabolizable energy and crude protein contents of the different experimental diets. Table 3.1 shows the ingredient composition of the five experimental diets.

The experimental diets were formulated, manufactured, and supplied by Voermol feeds (Maidstone Village, Tongaat, 4380, KwaZulu-Natal, South Africa). Each experimental diet was formulated and manufactured as one batch. All the feed was delivered at the same time and stored in a shed at the small stock section of the Hatfield Experimental Farm. The same feed was used for both the feedlot trial and the rumen fermentation study.

Table 3.1 Ingredient composition of the five experimental diets with different inclusion levels of urea and cottonseed oilcake meal.

	Treatment[1]				
Ingredients (%)[2]	**0% urea**	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**
Urea	0.00	0.42	0.83	1.25	1.66
Cotton seed oilcake meal	15.42	11.67	7.81	3.95	0.69
Maize meal	53.47	56.27	58.75	61.70	64.03
Sugarcane bagasse	11.76	12.55	13.43	14.32	15.08
Dried brewers grain	10.00	10.00	10.00	10.00	10.00
Molasses	6.00	6.00	6.00	6.00	6.00
Premix[3]	3.35	3.09	3.18	2.78	2.54

[1]Treatment: Percentage of urea included in experimental diets (as is basis)
Treatment 1 = 0%; Treatment 2 = 0.42%; Treatment 3 = 0.83%;
Treatment 4 = 1.25%; Treatment 5 = 1.66%

[2]Ingredients (%): Ingredient composition of experimental diets on an as is basis in %

[3]A standard premix supplied (final mix): 0.5% ammonium chloride; 0.45% salt; 0.9-1.1% calcium; 0.3-0.4%phosphorous; trace minerals and Vitamin A and 18ppm Monensin-Na

2.3 Animals, management and housing

The feedlot trial consisted of 260 South African Mutton Merino (SAMM) lambs which were sourced from three different breeder farms. The group consisted of 125 ewes, 99 wethers, and 36 rams. On the day of arrival, lambs were placed in a large pen to rest overnight, where they had *ad libitum* access to milled *Eragrostis curvula* hay and fresh drinking water.

On the second day, animals were individually weighed and processed. Lambs were assigned individually with a unique three-digit ear tag using a total tagger applicator (Allflex®). Each animal was weighed twice using a Tal-Tec (model TT40) livestock scale and the average weight of each lamb was recorded, which was used for blocking the animals when assigning them to treatments, as described in section 2.1. Lambs were vaccinated against clostridial and viral diseases (Multivax®, Swamycin® LA, Cydectin® LA) using an injection gun (HSW Roux-Revolver®) and treated against internal parasites (Ex-A-Lint®), which was all administered according to the instructions on the bottles. The animals did not receive hormonal growth implants.

On the third day, lambs were assigned to blocks, pens, and treatments as described in section 2.1. Lambs were tagged with a second ear tag, with the color of the tag corresponding to their allocated treatment. The colors were red for treatment 1; blue for treatment 2; pink for treatment 3; purple for treatment 4 and green for treatment 5. Of the 260 lambs, ten were outliers based on their weights and were culled from the study. They were kept in a separate pen to be used as replacement animals in the case of morbidity or mortality.

Lambs were then placed in their assigned pens, where they began with a gradual adaptation to their allocated treatment diets. The pens were approximately 13m x 4.5m in size, with concrete floors, which allowed 5.85m^2 floor space per lamb to reduce social stress and the number of shy feeders (Duddy *et al.,* 2016). Feed troughs were 4.5m long, which allowed 45cm feeding space per animal. Troughs were cover with a roof that extended into feedlot to allow sufficient space for animals to shelter during unfavorable weather conditions. Self-filling concrete water troughs were on the opposite side as the feeding troughs to prevent contamination of water (Duddy *et al.,* 2016).

The trial consisted of three days for arrival and processing, 14 days for adaptation, 54 days on feed, and one day for transport and slaughtering of lambs.

2.4 Adaptation phase

The lambs were adapted and transitioned onto a feedlot TMR over a period of 14 days to allow for efficient and successful adaptation. The importance of this adjustment period results from the fact that it takes approximately 10 days for the rumen microbes to adjust to any drastic changes in the level of concentrates. For the first 10 days, lambs received milled *Eragrostis curvula* hay *ad libitum* in addition to their allocated treatment diet. Lambs started with 100-gram pellets per lamb per day, which was increased daily with 100g/ lamb until *ad libitum* levels were reached (3.5 – 4.5% of body weight), at which stage lambs started to leave some pellets in feed troughs. The lambs were fed twice a day in two even portions. One half was fed at 08h00 and the other half at 15h00.

2.5 Feedlot phase

Animals were fed twice daily at 08h00 and 15h00 throughout the trial, and the amount offered per pen was recorded. This ensured animals had *ad libitum* access to fresh pellets at all times. Feed troughs were never empty, but feed bunk management ensured no build-up of pellets inside feed troughs as the feed then becomes unpalatable and moldy. Once every seven days the orts were weighed back, the weight was recorded and discarded. The weekly feed intake for each pen was determined

by subtracting the left-overs from the amount offered for the week. Weekly weigh-back was done every week at the same time.

Once every 7th-day lambs were weighed using the Tal-Tec (model TT40) livestock scale and each lambs' weekly weight was recorded. Pens were taken to the crush individually, and they were taken back to their allocated pen immediately after weighing, to minimize stress and time away from feed troughs. The weekly individual weights were averaged to get a weekly pen average weight. This weight was used to determine average daily gain (ADG) and together with weekly feed intake, the weekly feed conversion ratio (FCR) for each pen was calculated.

Water troughs were cleaned as needed and always provided enough fresh drinking water. Daily activities were kept to a minimum and as close as possible to disturb animals as little as possible. Pens were patrolled in the morning and afternoon to detect any visible signs of discomfort or other metabolic disturbances. Any sick or injured animals were treated and closely monitored until complete recovery. Feces were also observed for any signs of diarrhea, digestive disturbances, or the presence of internal parasites.

2.6 Feed sampling

For 10 weeks, once every seven days, ±750g feed samples were collected. For each treatment, grab samples were taken from different locations inside six different bags, which were then pooled per treatment per week. Samples were placed in labeled (treatment number and date) air-tight sealed plastic bags which were stored at –20^{o}C for further analysis. Before laboratory analysis, ten weekly feed samples were pooled and mixed into one composite sample, and a representative sub-sample was taken for laboratory analysis. Feed samples were analyzed at the UP Nutrilab (University of Pretoria, Pretoria, Gauteng) according to the procedures of the AOAC (2000) and VanSoest *et al*. (1997).

2.7 Slaughtering and carcass data

After 54 days on feed, the lambs were weighed twice the day before slaughter and the average of the two weights was taken as the final live body weight. Lambs were transported the next morning to Diamond L Abattoir; PO Box 24, Welbekend, 1517, where they were all slaughtered on the same day. Lambs were slaughtered by making use of the New Zealand method of inverted slaughtering and then carcass data was obtained. The Wairoa process is a technique of slaughtering developed by New Zealand, which involves an electrical head-only stunning. This renders the animal insensitive to pain but able to recover if the slaughter cut is not made. The heart

remains beating. The system is humane, safe for workers, and generally accepted asHalal by Muslims.

2.8 Parameters measured

The following parameters were monitored and calculated using the data collected during the feedlot phase, slaughtering process, and laboratory analysis.

Performance data and feed intake

- Pen average body weights (weekly and overall)
- Pen average daily gain (ADG) (weekly and overall)
- Pen average daily feed intake (ADFI) (weekly and overall)
- Pen feed conversion ratio (FRC) (weekly and overall)

Carcass parameters

- Cold carcass weight (kg)
- Dressing percentage (%)

2.9 Sample analysis

Laboratory analysis was done at Nutrilab, Department of Animal and Wildlife Sciences, University of Pretoria. Feed samples were analyzed in duplicate for DM (procedure 934.01 AOAC, 2000), CP was analyzed using Leco analysis (procedure 968.06 AOAC, 2000), EE (procedure 920.39 AOAC, 2000), NDF (Van Soest & Robertson, 1997), starch (procedure 996.11 AOAC, 2000), GE (ASTMD2015), ash (procedure 942.05 AOAC, 2000), calcium (Giron, 1973), phosphorus (procedure 965.17 AOAC, 2000), and *In vitro* organic matter digestibility (*IVOMD*) as described by Tilley & Terry (1963) modified by Engels & Van der Merwe (1967).

2.10 Statistical analysis

The data was analyzed statistically as a randomized complete block design with the GLM model (Statistical Analysis System, 2019) for the average effects over time. Repeated Measures Analysis of Variance with the GLM model was used for repeated period measures. Means and standard error were calculated and the significance of difference (P <0.05) between means was determined by Fischer's test (Samuels, 1989). The starting live body weight was included as a covariate against the average daily gain and final live body weight.

The linear model used is described by the following equation:

$y_{ij} = \mu + T_i + \rho_j + e_{ij}$

y_{ij} = response due to experimental unit with the i^{th} treatment in the j^{th} block

μ = overall mean

T_i = the treatment effect

ρ_j = the block effect (represents the average deviation of units in block j from the overall mean)

e_{ij} = assumed to be independent with zero means and common variance

Linear and quadratic relationships between the urea percentages of the treatment diets and the dependent variables were determined in a multivariate analysis with the GLM model (Statistical Analysis System, 2019).

3. Results and discussion

3.1 Introduction

To assess the effect of level of urea supplementation and protein qualityon the growth performance of lambs, a feedlot trial was conducted in which lambs received experimental diets which differed in inclusion levels of urea. In the followingsections the results obtained during the feedlot trial, slaughtering process, and laboratory analysis will be discussed and compared with other results obtained from similar studies.

3.2 Chemical composition of experimental diets

The pooled feed samples of the five experimental diets were analyzed and the chemical composition of each treatment diet is shown in Table 3.2. These analytical results of the experimental diets will apply to Chapter 4 as well since the same batch of feed was used in both experiments.

The ingredients used in the formulation of these diets were representative of the typical ingredients used in South African feedlot diets (Smith, 2008; Brand *et al.,* 2013; Van de Vyver *et al.,* 2013; Brand *et al.,* 2017; O'Reilly, 2018). The main difference was the roughage source used in this trial (O'Reilly, 2018). The typical roughage source used in sheep feedlot diets is lucerne, however, in this trial sugarcane bagasse was used as seen in Table 3.1. Bagasse is a high fiber residue remaining after the sugarcane stalk has been crushed and the juice extracted (Pandey *et al.,* 2000).

As shown in Table 3.2, diets were formulated on an iso-nitrogenous, iso-energy, and iso-fibrous basis, so that the effects of different inclusion levels of urea could be investigated. There was relatively little variation between diets in their nutrient composition, except for the starch levels. As urea inclusion levels increased there were lower inclusion levels of CSOCM and higher inclusion levels of maize meal. This can explain the increasing levels of starch in the diets as the urea levels increased. The effects of the increasing levels of starch will be discussed later.

Table 3.2 Chemical composition (%DM) of the five experimental diets differing in inclusion levels of urea.

	Treatment[1]				
Nutrients[2]	**0% urea**	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**
Dry matter (g/kg)	90.36	89.50	89.90	89.79	89.93
Organic matter (%DM)	91.76	92.07	91.05	92.96	93.50
Crude protein (%DM)	14.93	15.52	15.49	15.75	15.18
[3]Gross energy (MJ/kg DM)	17.76	17.60	17.27	17.38	17.30
[4]Metabolisable energy (MJ/kg DM)	11.11	11.18	10.92	11.17	11.21
Starch (%DM)	34.74	35.83	37.74	41.80	42.10
Neutral detergent fibre (%DM)	29.71	27.72	29.39	25.06	25.70
Ether extract (%DM)	2.54	2.85	2.64	2.40	2.47
Ash (%DM)	8.24	7.93	8.95	7.04	6.50
Calcium (%DM)	0.88	1.16	1.13	1.19	0.96
Phosphorus (%DM)	0.34	0.37	0.44	0.38	0.30
[5]*IVOMD* (%DM)	76.27	77.48	77.11	78.39	79.03

[1]Treatment: Percentage of urea included in experimental diets (as is basis)
Treatment 1 = 0%; Treatment 2 = 0.42%; Treatment 3 = 0.83%;
Treatment 4 = 1.25%; Treatment 5 = 1.66%

[2]Nutrients in experimental diets determined by proximate analysis

[3]Gross energy (MJ/kg) - Determined using bomb calorimetry

[4]Metabolisable energy (MJ/kg) = 0.82 x (GE x *IVOMD*) (Robinson *et al.,* 2004)

[5]*IVOMD* - *In vitro* organic matter digestibility

3.3. The effect of different inclusion levels of urea on growth performance, feed intake and feed efficiency

Table 3.3 summarises the overall growth performance and feed efficiency of feedlot lambs obtained over 54 days feeding period. Table 3.4 summarises the linear and quadratic relationships between the urea percentages and the dependent variables.

Table 3.3 The effect of different inclusion levels of urea on mean (±SE) growth performance and feed efficiency of feedlot lambs.

	Treatment					
Parameters	**0% urea**	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**	**±SE[1]**
Growth performance						
Starting body weight (kg)	31.2^{ab}	31.6^{a}	30.6^{c}	30.7^{bc}	30.5^{c}	0.26
Final body weight (kg)	47.9^{a}	47.8^{a}	45.8^{b}	45.8^{b}	46.1^{b}	0.57
Weight gained (kg)	16.7^{a}	16.2^{ab}	15.2^{b}	15.2^{b}	15.6^{ab}	0.49
ADG (kg/lamb/day)	0.309^{a}	0.299^{ab}	0.281^{b}	0.282^{b}	0.290^{ab}	0.0091
Feed efficiency						
Dry matter intake (kg)	1.762^{a}	1.756^{ab}	1.685^{bc}	1.661^{c}	1.630^{c}	0.0247
FCR	5.70^{a}	5.87^{ab}	5.99^{b}	5.92^{ab}	5.63^{a}	0.136

^{a,b,c} Row means with different superscript differ significantly (P <0.05)

^{d,e} Row means with a different superscript tend to differ (P <0.10)

[1]±SE: Standard error

Table 3.4 The linear and quadratic relationships between the different urea inclusion levels and the dependent variables.

Variable	Linear relationship	*P*-value	Quadratic relationship	*P*-value
Starting body weight	y = -0.57x + 31.38	0.112	$y = -0.01x^2 - 0.56x + 31.37$	0.376
Final body weight	y = -1.35x + 47.80	0.087	$y = 1.15x^2 - 3.25x + 48.20$	0.205
Weight gained	y = -0.77x + 16.42	0.128	$y = 1.15x^2 - 2.69x + 16.82$	0.086
ADG	y = -0.13x + 0.303	0.158	$y = 0.02x^2 - 0.05x + 0.311$	0.082
Dry matter intake	y = -0.09x + 1.77	**0.006**	$y = 0.001x^2 - 0.08x + 1.77$	0.055
FCR	y = -0.02x + 5.84	0.883	$y = -0.46x^2 + 0.74x + 5.68$	**0.036**

3.3.1. Growth performance

Starting live body weight

The mean starting live body weight of lambs at the beginning of the feedlot trial was 30.9 kg, which is similar to starting body weights of similar feedlot studies conducted in South Africa (Price *et al.,* 2009; Van de Vyver, 2013). The starting live body weight of the 0.42% urea treatment was higher (P <0.05) than the starting live body weights of lambs in the 0.83% urea treatment, 1.25% urea treatment, and the 1.66% urea treatment, with the 0.42% urea treatment having the highest starting body weight of 31.6 kg. The 0% urea treatment had a higher starting live body weight (P <0.05)than the 0.83% and 1.66% urea treatments. Differences and tendencies had no effecton results, as the starting live body weight was included as a covariate against ADG and final live body weight.

Body weight gain

The mean body weight gained during the 54day feedlot period was 15.8 kg, with values ranging from 16.7 kg to 15.2 kg as seen in Table 3.3. This is in agreement with results reported by Sheridan *et al.* (2003) of 15.7 kg for South African Mutton Merinos fed over a period of 56 days. Van de Vyver (2013) reported values ranging from 12.50 – 15.25 kg body weight gain during a study in which Merino lambs were finished on diets differing in inclusion levels of maize silage over 60 days.

There were differences in body weight gain (P <0.05) between the 0% urea treatment and the 0.83% and 1.25% urea treatments, with lambs in the 0% urea

treatments having greater body weight gain. There was no difference (P >0.05) in body weight gain between 0% and 0.42% urea treatments and the treatment with the highest urea inclusion (1.66% urea). There tended (P <0.10) to be a quadratic relationship between urea inclusion levels and weight gain as seen below in Figure 3.2.

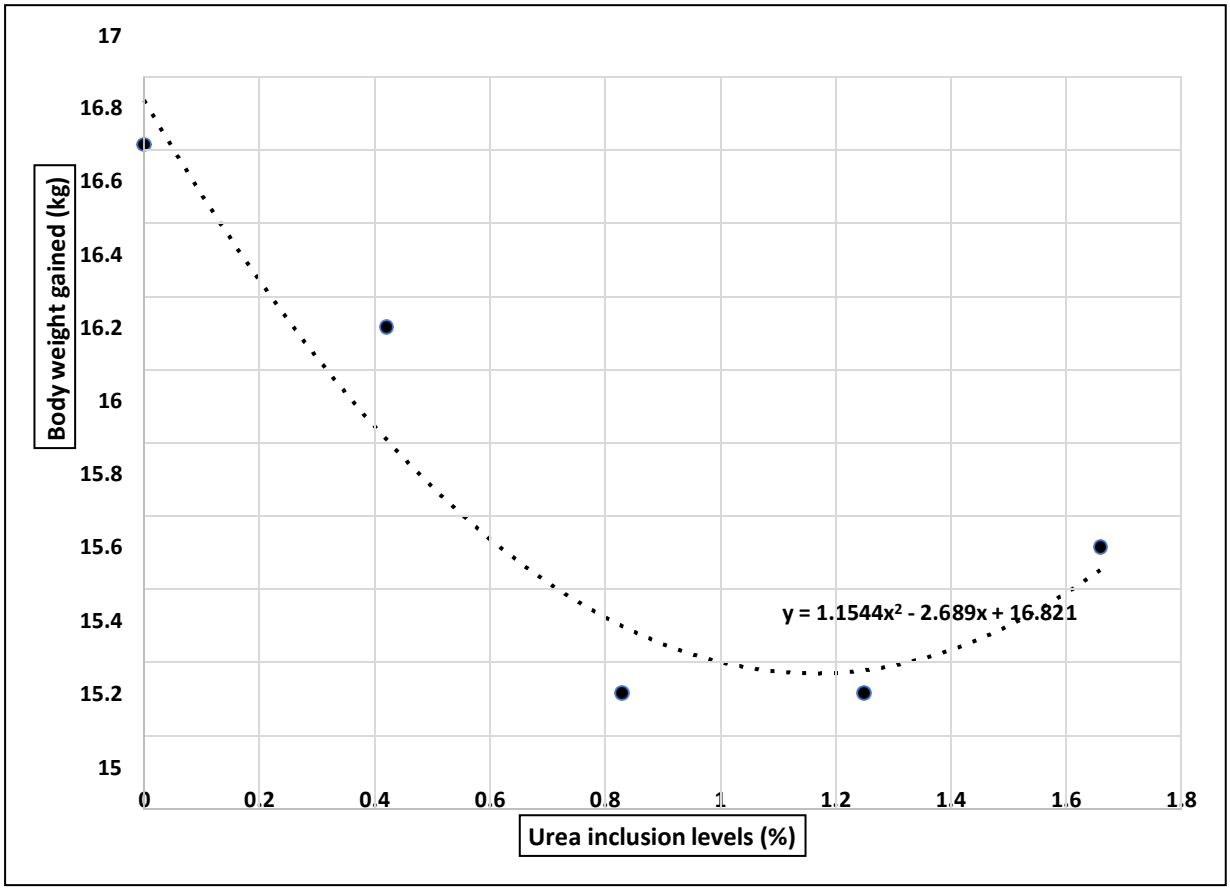

Figure 3.2 The quadratic relationship (P <0.10) between urea inclusion levels and body weight gained.

Average daily gain

Table 3.5 The cumulative average daily gains of feedlot lambs at different stages of the trial.

	Treatment					
	0% urea	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**	**±SE[1]**
ADG (kg)						
Day 1-14	0.374^{a}	0.340^{ab}	0.316^{ab}	0.286^{b}	0.306^{b}	0.0214
Day 1-21	0.331^{a}	0.301^{ab}	0.276^{bc}	0.257^{c}	0.298^{abc}	0.0139
Day 1-28	0.351^{a}	0.345^{ab}	0.330^{ab}	0.312^{b}	0.321^{ab}	0.0121
Day 1-35	0.350^{a}	0.330^{ab}	0.312^{b}	0.306^{b}	0.318^{ab}	0.0112
Day 1-42	0.338^{a}	0.322^{ab}	0.316^{ab}	0.301^{b}	0.308^{b}	0.0085
Day 1-49	0.317^{a}	0.310^{ab}	0.288^{b}	0.287^{b}	0.295^{ab}	0.0088
Day 1-54	0.309^{a}	0.299^{ab}	0.281^{b}	0.282^{b}	0.290^{ab}	0.0091

^{a,b,c} Row means with different superscript differ significantly (P <0.05)

^{1}±SE: Standard error

The mean average daily gain for this trial was 0.292 kg/lamb/day, with values ranging from 0.281 - 0.309 kg/lamb/day. The 0.83% urea treatment had the lowest ADG of 0.281 kg/lamb/day, which is in agreement with results reported of 0.281 kg/lamb/day (Sheridan *et al.,* 2003). Brand *et al.* (2017) reported an ADG for South African Mutton Merinos over 63 days in feedlot of 0.274 kg/lamb/day and 0.336 kg/lamb/day for two groups respectively. Price *et al.* (2009) reported higher ADG's, which ranged from 0.298 – 0.340 kg/lamb/day, but these lambs received a hormonal implant which could explain the higher values obtained. Pienaar *et al.* (2012) recorded anADG of 0.330 kg/lamb/day for South African Mutton Merinos lambs on a finishing diet.

Overall the ADG was highest between day 22–28 with a mean of 0.449 kg/lamb/day as seen from Figure 3.4, this is in agreement with ADG values of 0.440 kg/lamb/day obtained from South African Mutton Merinos after 21 days in feedlot (Brand *et al*., 2017). During day 15-21 there was heavy rainfall, which may have resulted in the lower ADG (0.240 kg/lamb/day) for that feeding period, although the DMI was not affected by the weather conditions. This in turn may have resulted in high ADG values achieved in the next seven days as a result of compensatory growth. The average daily gains of lambs receiving the 0% urea treatments were significantly higher (P <0.05) than the 0.83% and 1.25% urea treatments.

The results of this study are in agreement with other related studies. Thomas *et al.* (1984) reported a 12% higher ADG for steers receiving an iso-nitrogenous diet supplemented with soybean meal compared with a diet supplemented with 0.93% urea. Zinn *et al.* (2000) reported a 17% higher ADG for cattle receiving a diet

containing 20% NPN compared with cattle receiving a diet containing 40% NPN. The higher ADG obtained in this trial from lambs receiving diets supplemented with only CSOCM (0% urea diet) is in agreement with results reported by other studies when only natural protein was supplemented (Huston & Shelton., 1971 & Milton *et al.,* 1997).

Contradicting to other studies with incremental levels of urea supplementation (Huston & Shelton., 1971 & Milton *et al.,* 1997), is the fact that the 0% urea and 1.66% urea treatments had no difference in ADG obtained for the study, even though 0% urea treatments had higher DMI. This can be explained by the better FCR of lambs receiving the 1.66% urea treatments (Table 3.3), therefore no differences were obtained in the growth performance of the lambs receiving 0% and 1.66% urea treatments.

Another point of discussion is the large difference in ADG values between the different treatments for the first 21 days on feed. As seen from Figure 3.3 the difference in ADG values for the different treatments is becoming less towards the end of the trial. This is why there is a perception that protein quality is not that important in the finishing diets of lambs, because of the compensatory growth of lambs towards the end of the feeding period. After 14 days on feed, the 0% urea treatment had the highest ADG (0.374 kg/lamb/day), which was significantly higher (P <0.05) than the 1.25 and 1.66% urea treatments. Throughout the remainder of the trial, the 0% urea treatment had the highest ADG. On day 49 the 0% urea treatment was no longer significantly higher (P >0.05) than the 1.66% urea treatment, but the 0% urea treatment was higher (P <0.05) than the 0.83% urea treatment. The reason for this is not clear. From day 14 (first weighing) until day 54, the 1.25% urea treatment was lower (P <0.05) than the 0% urea treatments.

On day 21 of the feeding period, the treatment with the highest ADG (0% urea treatment) was 22.36% higher than the treatment with the lowest ADG (1.25% urea treatment), but at 54 days on feed, the difference between the highest ADG (0% urea) and lowest ADG (0.83% urea) was only 9.06%. This is in agreement with other studies (Meiske & Goodrich, 1966). Sindt *et al.* (1994) reported a significant difference in weight gain for calves receiving soy bean meal as opposed to urea and feather meal, but over the entire study (117days) there were no differences. Maximum utilization ofurea by lambs fed a semi-purified diet occurred after 35 days in a study conducted by Welch *et al.* (1957). Several authors have reported an increase in nitrogen retention as the feeding period progresses (Repp *et al.,* 1955a; Anderson *et al.,* 1959; Smith *et al.,* 1960). There tended (P <0.10) to be a quadratic relationship between urea inclusion levels and ADG as seen in Figure 3.5.

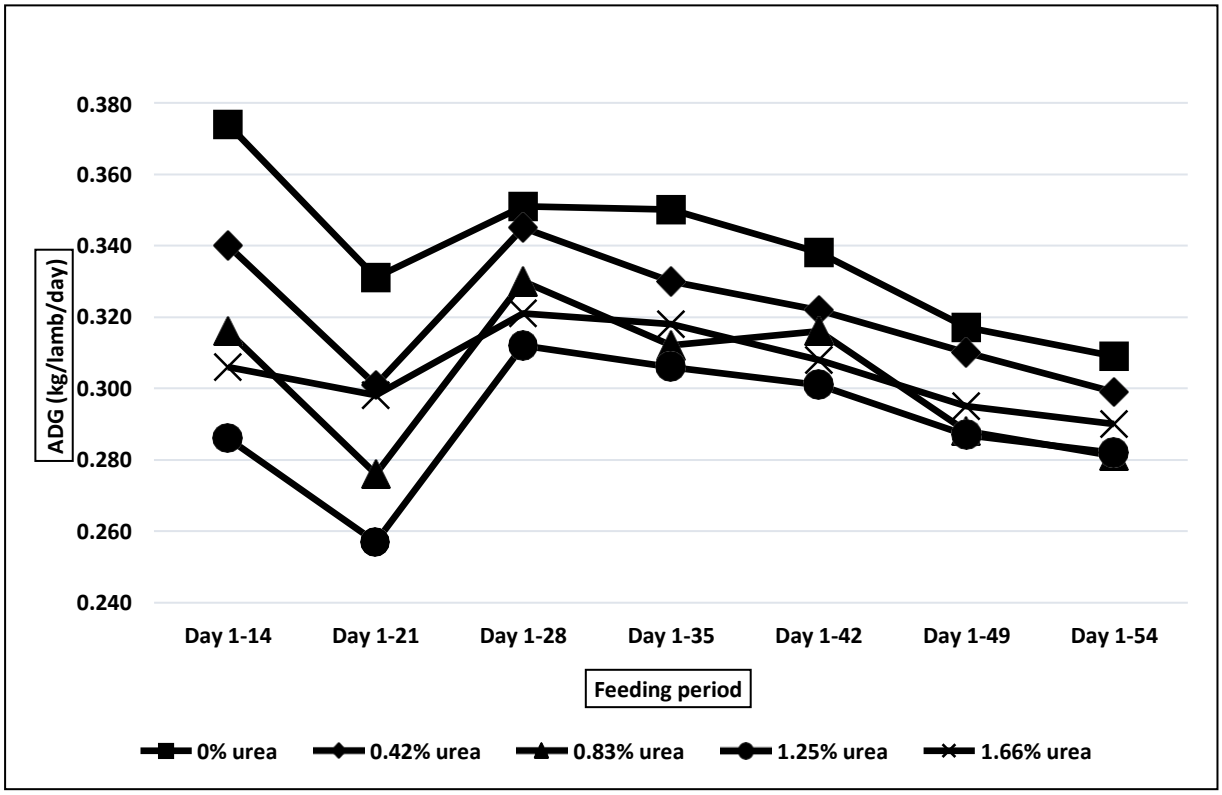

Figure 3.3 The cumulative average daily gains of feedlot lambs fed different levels of urea.

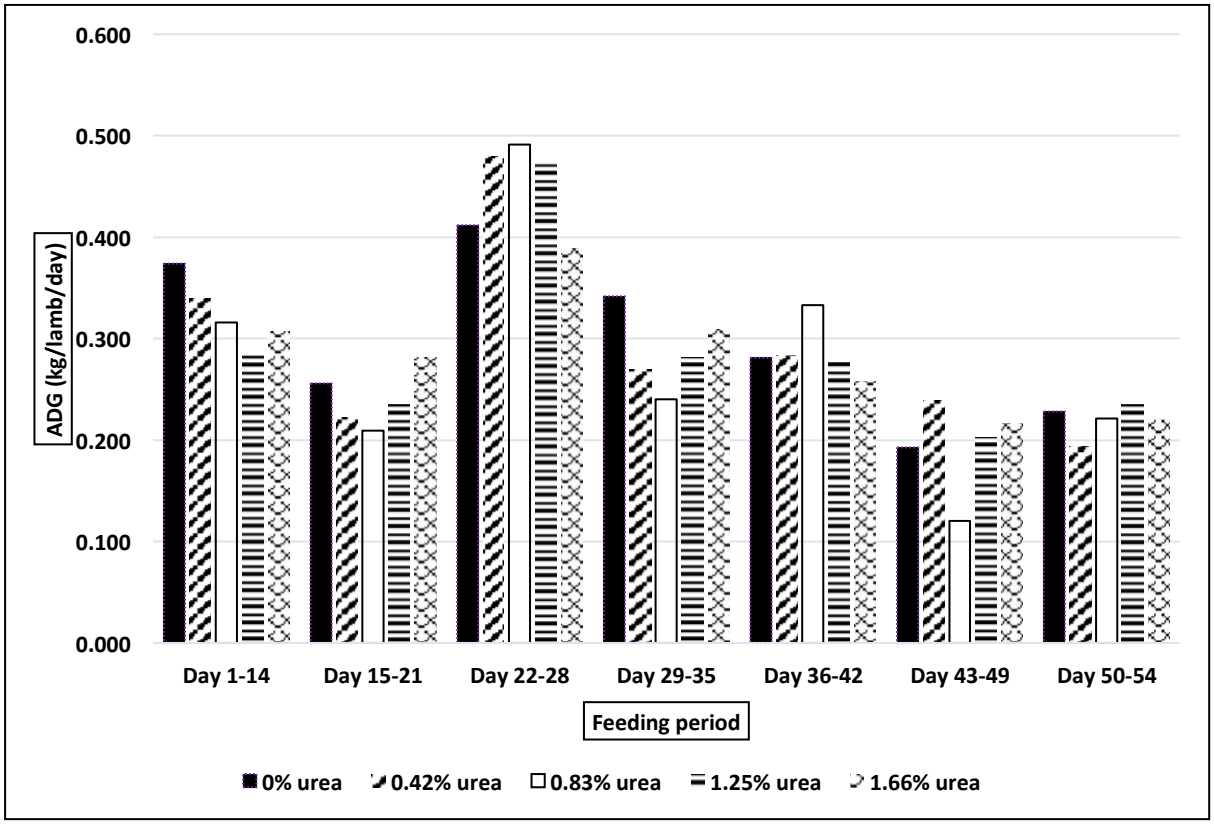

Figure 3.4 Average daily gain of feedlot lambs fed different levels of urea over a 54day feedlot period.

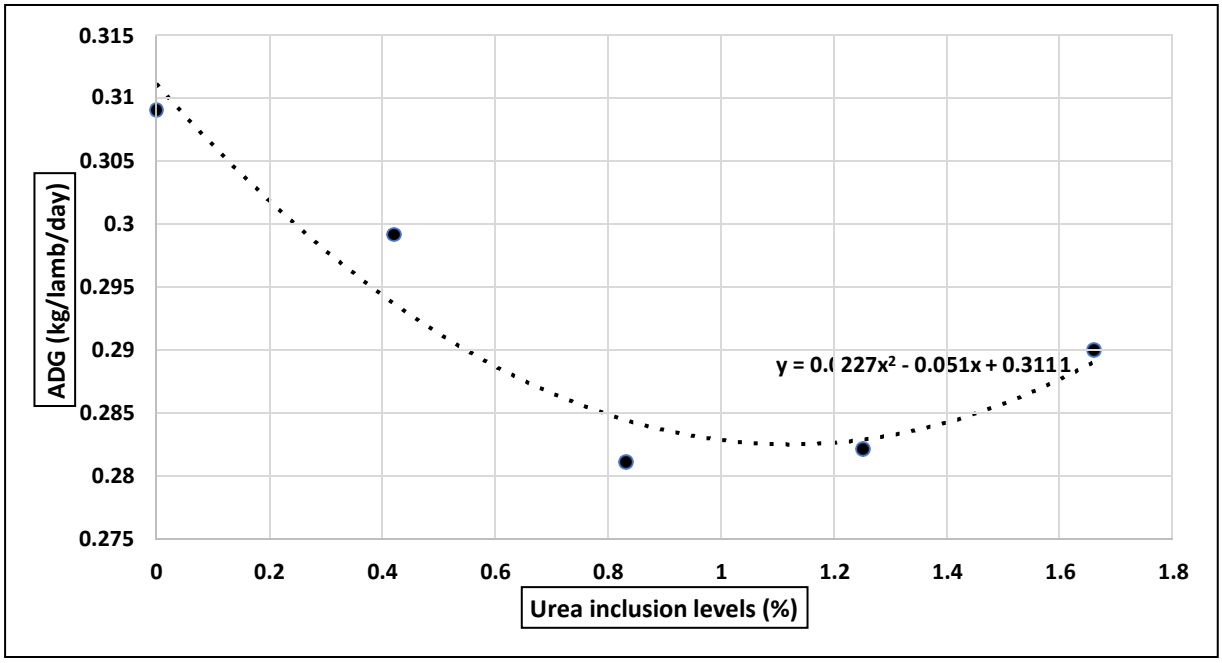

Figure 3.5 The quadratic relationship ($P < 0.10$) between urea inclusion levels and average daily gain.

3.3.2. Feed efficiency

Dry matter intake (DMI)

Table 3.6 The cumulative average dry matter intake of feedlot lambs at different stages of the trial.

	Treatment					
	0% urea	0.42% urea	0.83% urea	1.25% urea	1.66% urea	±SE[1]
ADG (kg)						
Day 1-14	1.571^{a}	1.465^{ab}	1.412^{bc}	1.417^{bc}	1.348^{c}	0.0236
Day 1-21	1.593^{a}	1.542^{a}	1.477^{b}	1.475^{b}	1.440^{b}	0.0191
Day 1-28	1.654^{a}	1.611^{ab}	1.555^{bc}	1.535^{c}	1.497^{c}	0.0201
Day 1-35	1.688^{a}	1.644^{ab}	1.582^{bc}	1.570^{c}	1.538^{c}	0.0228
Day 1-42	1.704^{a}	1.668^{ab}	1.619^{bc}	1.593^{c}	1.566^{c}	0.0225
Day 1-49	1.716^{a}	1.696^{ab}	1.635^{bc}	1.611^{c}	1.580^{c}	0.0238
Day 1-54	1.762^{a}	1.756^{ab}	1.685^{bc}	1.661^{c}	1.630^{c}	0.0247

^{a,b,c} Row means with different superscript differ significantly (P <0.05)

[1]±SE: Standard error

The mean dry matter intake (DMI) for this trial was 1.698kg/lamb/day, with values ranging from 1.629 – 1.763 kg/lamb/day. Pienaar *et al.* (2012) observed DMI in South African Mutton Merino lambs on a finishing diet to be 1.60 kg/lamb/day. Sheridan *et al.* (2003) reported an average DMI of 1.605 kg/lamb/day. Brand *et al.* (2017) also reported DMI for one group of Merino lambs and two groups of South African Mutton Merinos. The Merino lambs had a DMI of 1.494 kg/lamb/day, compared with the higher intakes of the two South African Mutton Merino groups of 1.674 and 1.635 kg/lamb/day respectively.

Lower DMI values were reported by Price *et al.* (2009) of 1.380 kg/lamb/day. In that study, the feed was not offered in pelleted form, which could explain the lower intake value recorded. Feeding of pelleted feed increased the ADG when compared with diets in non-pelleted form (Casey & Webb, 1995). Lambs cannot select the more palatable feed components from the pelleted feed, therefore higher intakes can be achieved, which in turn also influences the live weight of the lambs (Sheridan *et al.,* 2003). Van de Vyver *et al.* (2013) reported DMI values ranging from 0.91 – 1.57 kg/day for Merinolambs with varying levels of maize silage being included in the diets. Maize silage is a bulky wet feed that contributed to the lower DMI.

There was a difference (P <0.05) in DMI between the 0% urea and the 0.83%, 1.25%, and 1.66% urea treatments, as well as a difference (P <0.05) between the 0.42% urea treatment when compared with the 1.25% and 1.66% urea treatments, with the 0% and 0.42% urea treatments having higher DMI. This suggests that the higher inclusion levels of urea did affect the palatability of the diet, and therefore DMI reduced as urea inclusion levels increased. As urea supplementation was increased in the diets, there was a linear decrease (P <0.05) in DMI as seen in Figure 3.7. As seen in Figure 3.6, this linear decrease in DMI as urea supplementation increased was evident throughout the trial. Almost all reports on reduced animal production when urea is fed are attributable to a reduction in feed intake due to palatability (Polan *et al.,* 1976; Kertz, 2010). Broderick *et al.* (1993) did not observe a significant reduction in feed intake when replacing natural protein with 1.33% urea but did observe a significant reduction when urea was included at 1.63%.

In this study, urea replaced the natural plant protein, cottonseed oil cake meal (CSOCM). Huston & Shelton (1971) as cited by Stanton & LeValley (2006) reported similar results with higher DMI values when lambs received CSOCM compared with CSOCM plus urea as their dietary protein source, with values of 1.48 and 1.41 kg/lamb/day respectively. Milton *et al.* (1997) reported 4% and 10% higher DMI values for steers receiving a diet supplemented with soya bean meal and CSOCM respectively, compared with a urea supplemented diet. In Figure 3.6 it is noted that all treatments had increasing DMI with days in feedlot. Brand *et al.* (2017) reported increasing feed intake in lambs with an increase in age.

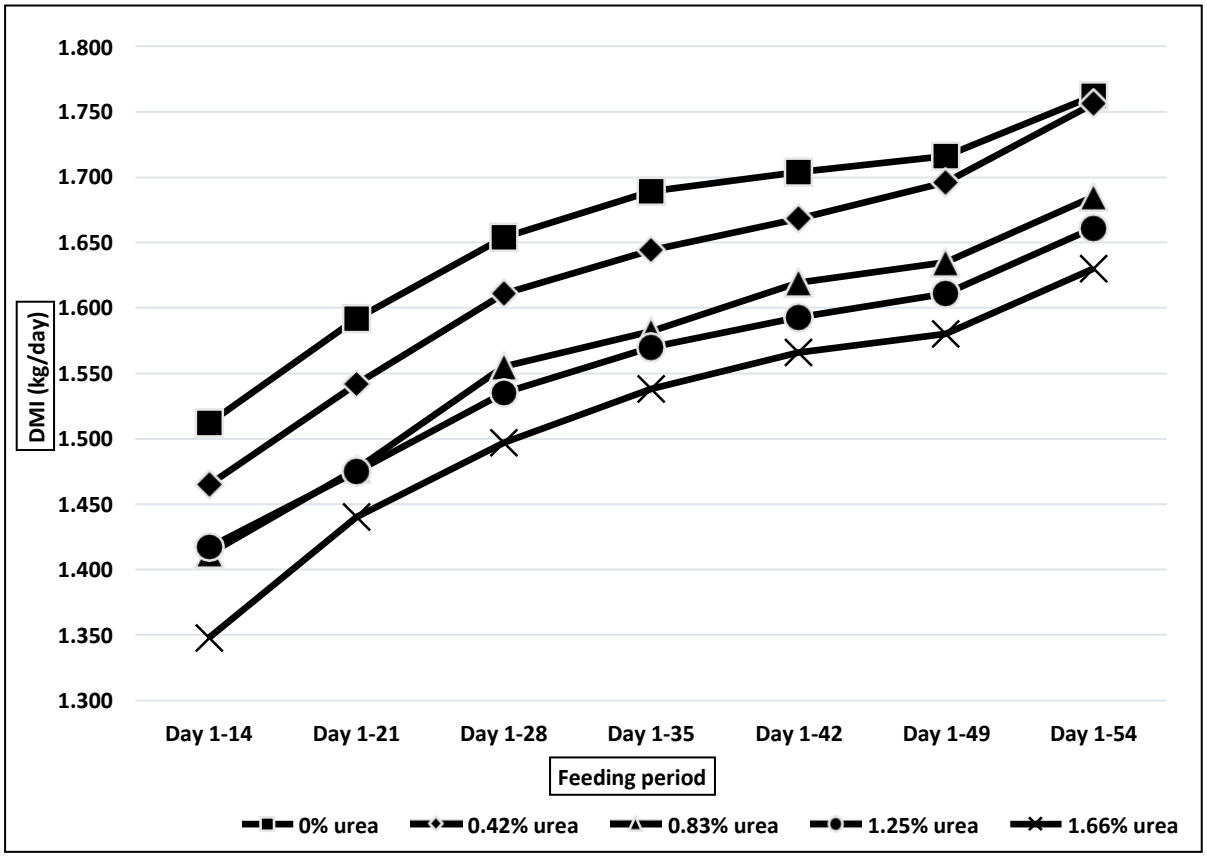

Figure 3.6 Effect of different levels of urea supplementation on mean DMI of feedlot lambs.

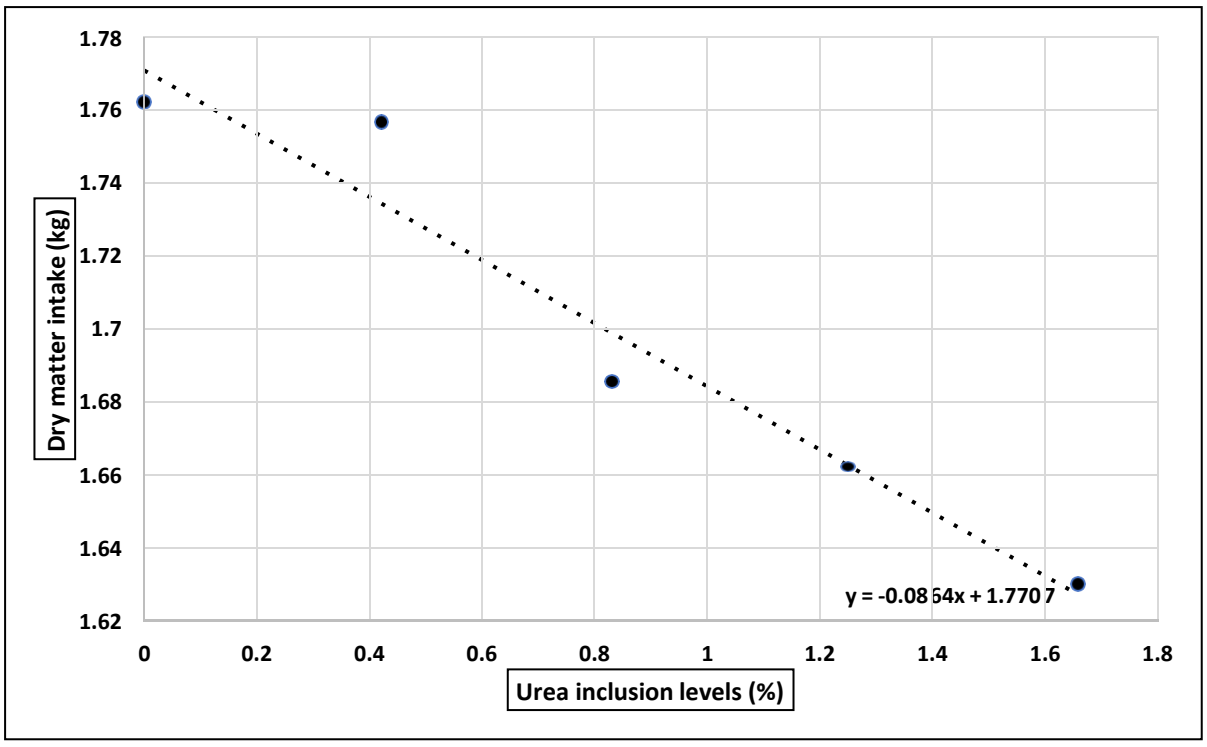

Figure 3.7 The linear relationship ($P < 0.05$) between urea inclusion levels and dry matter intake.

Feed conversion ratio (FCR)

Table 3.7 Feed conversion ratio of feedlot lambs at different stages of the trial.

	Treatment					
	0% urea	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**	**±SE[1]**
ADG (kg)						
Day 1-14	4.08[a]	4.35[ab]	4.49[ab]	5.08[b]	4.50[ab]	0.270
Day 1-21	4.82[a]	5.15[ab]	5.47[ab]	5.81[b]	4.84[ab]	0.243
Day 1-28	4.72[a]	4.67[a]	4.74[a]	4.97[a]	4.68[a]	0.152
Day 1-35	4.83[a]	4.99[a]	5.08[a]	5.19[a]	4.84[a]	0.139
Day 1-42	5.04[a]	5.18[a]	5.13[a]	5.32[a]	5.09[a]	0.107
Day 1-49	5.41[cd]	5.48[cd]	5.69[d]	5.66[d]	5.36[cd]	0.123
Day 1-54	5.70[a]	5.87[ab]	5.99[b]	5.92[ab]	5.63[a]	0.136

[a,b] Row means with different superscript differ significantly (P <0.05)

[c,d] Row means with a different superscript tend to differ (P <0.10)

[1]±SE: Standard error

The mean FCR for this trial was 5.81 with values ranging from 5.63 – 5.99. Price *et al.* (2009) reported lower FCR values of 4.51 – 4.74. In that trial, lambs received a hormonal implant (Zeraplix), which could explain the lower FCR values. Salisbury *et al.* (2007) obtained significant differences in FCR when lambs received Zeranol implants compared with control animals that had no implants. Sheridan *et al.* (2003) reported an FCR of 6.47 for South African Mutton Merinos over a 56day feedlot period. The FCR for this trial is representative of FCR values obtained from other South African feedlot studies. FCR values of 5.5 and 7.2 were reported for two groups of South African Mutton Merinos by Brand *et al.* (2017), Gouws *et al.* (2016) reported a mean FCR of
5.52 and Van de Vyver *et al.* (2013) reported a mean FCR for Merino lambs of 5.87.

Poor FCR values were obtained for the 0.83% urea treatment for days 15-21 and days 43-49 as seen in Figure 3.9. At the same feeding periods, there were no radical differences in DMI, but lower ADG values were obtained for the 0.83% urea treatment. The low ADG obtained across all treatment diets during days 15-21 explains the poor FCR values for that period. As with ADG, the poor average FCR value of 10.30 across all treatments for days 15-21, is followed by a good FCR value of 4.44 for the next feeding period. From days 1-21 the 0% urea treatment had a better FCR (P <0.05) than the 1.25% urea treatment. From days 22-42, there were no differences. At the end of the trial, the 0% and 1.66% urea treatments had a better (P <0.05) FCR than the 0.83% urea treatment. As seen in Figure 3.10 there was a quadratic relationship (P <0.05) between urea supplementation levels and FCR.

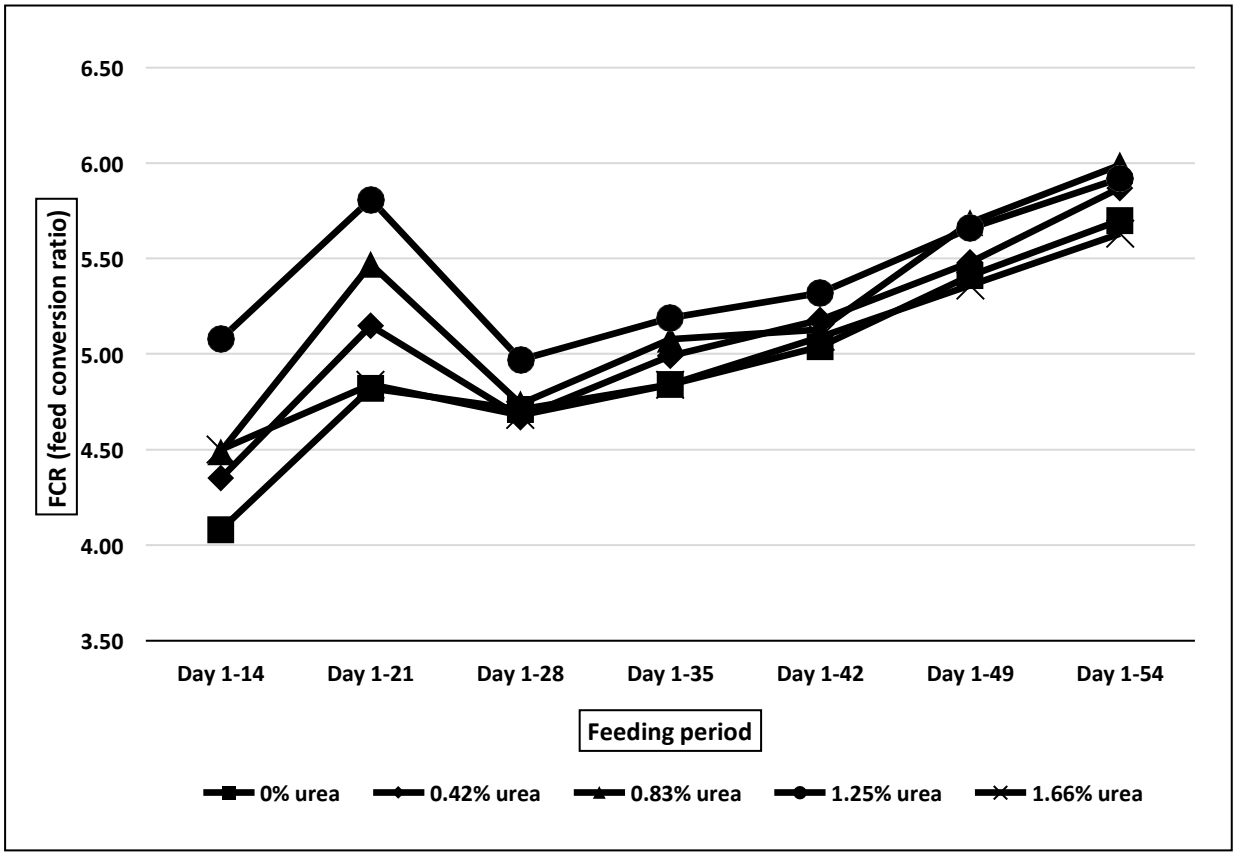

Figure 3.8 Feed conversion ratio of feedlot lambs fed different levels of urea.

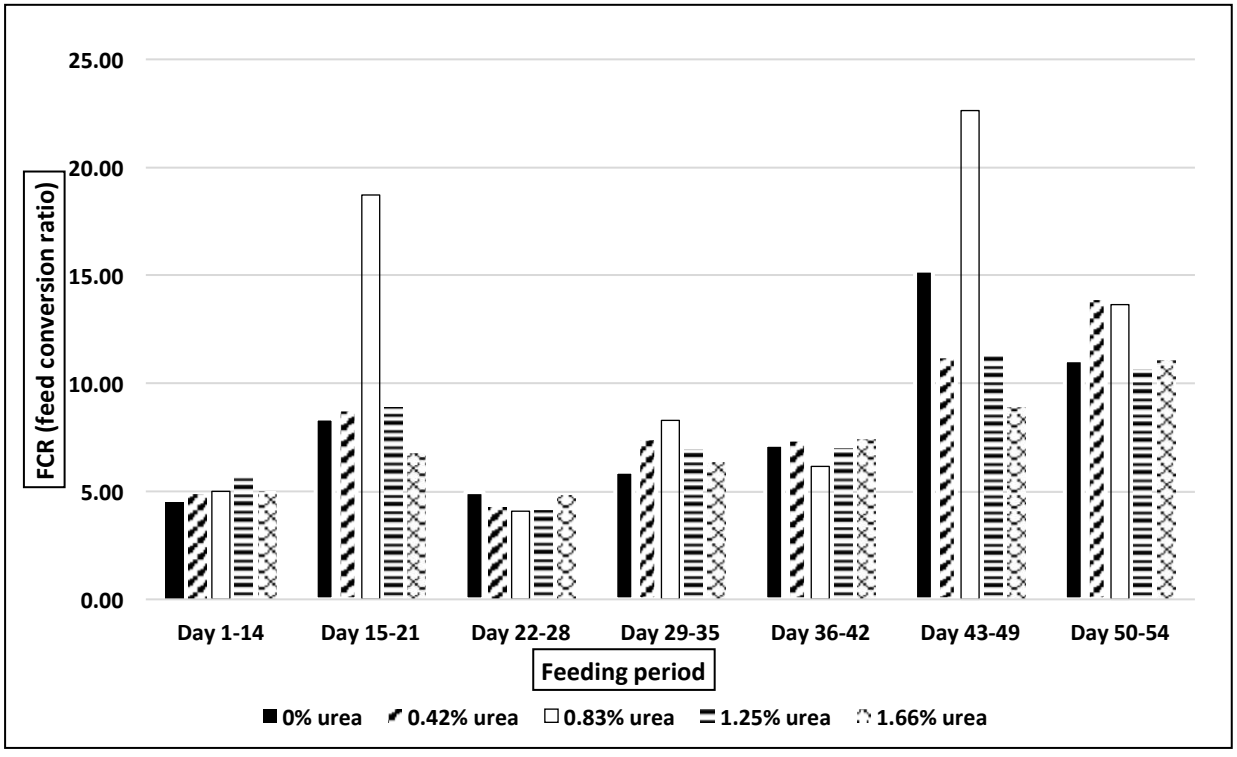

Figure 3.9 Feed conversion ratio of feedlot lambs fed different levels of urea over a 54day feedlot period.

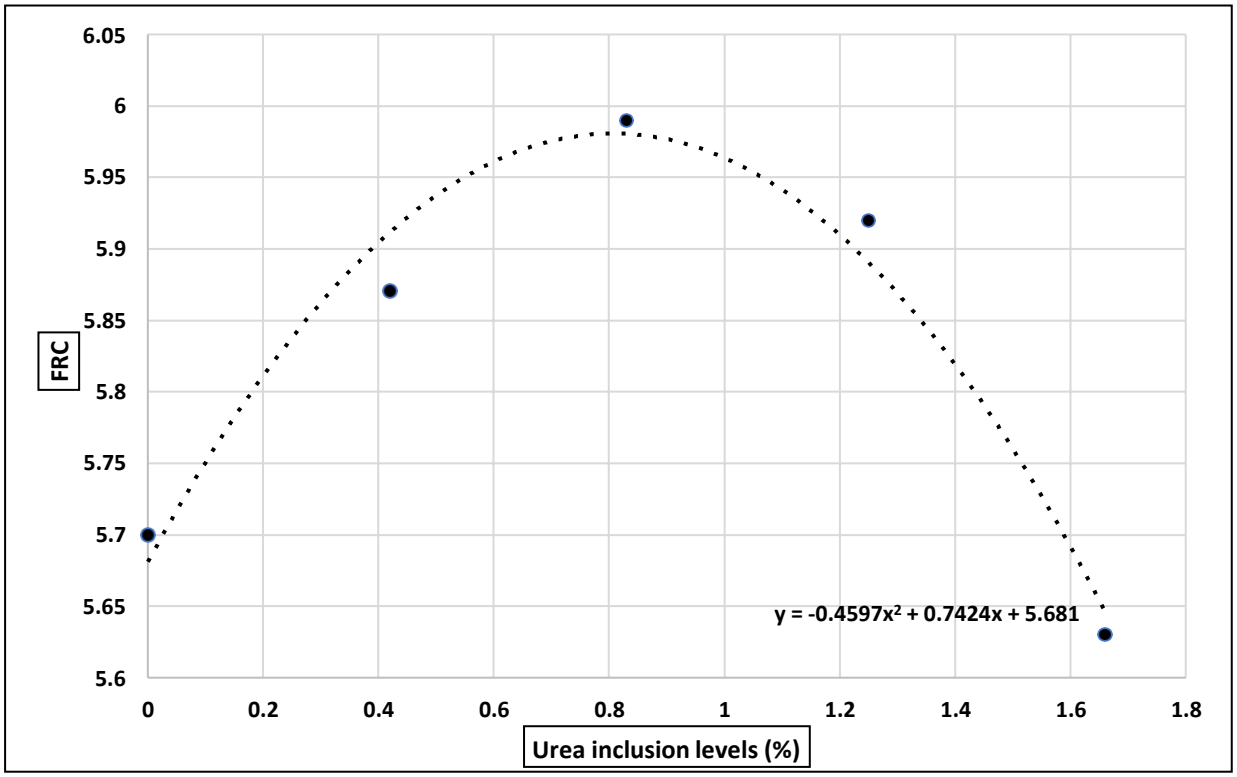

Figure 3.10 The quadratic relationship (*P* <0.05) between urea inclusion levels and feed conversion ratio.

3.4. The effect of different inclusion levels of urea on carcass parameters of feedlot lambs

Table 3.8 is the results of the carcass parameters recorded and calculated after the lambs were slaughtered. In Table 3.9 is the linear and quadratic relationship between the urea percentages and the dependent variables.

Table 3.8 The effect of different inclusion levels of urea on mean (±SE) carcass parameters of feedlot lambs.

	Treatment					
Parameters	**0% urea**	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**	**±SE[1]**
Cold carcass weight (kg)	24.66[a]	24.35[a]	23.25[b]	23.03[b]	23.41[b]	0.307
Dressing percentage (%)	48.35[cd]	48.57[c]	47.40[d]	47.63[cd]	47.51[d]	0.400

[a,b] Row means with the same superscript do not differ significantly (*P* >0.05)[c,d] Row means with a different superscript have a tendency (*P* <0.10) [1]±SE: Standard error

Table 3.9 The linear and quadratic relationships between the different urea inclusion levels and the dependent variables.

Variable	Linear relationship	*P*-value	Quadratic relationship	*P*-value
Cold carcass weight	y = -0.92x + 24.51	0.075	$y = 0.93x^2 - 2.46x + 24.83$	0.123
Dressing percentage	y = -0.63x + 48.42	0.120	$y = 0.29x^2 - 1.11x + 48.51$	0.361

Carcass weights

The recorded cold carcass weights of each treatment are shown in Table 3.8. The mean cold carcass weight of lambs was 23.7 kg for this trial, with values ranging from 24.66 kg to 23.03 kg. These carcass weights are in agreement with the weights reported by Gouws *et al.* (2016) of 23.1 kg. Brand *et al.* (2017) reported a lower carcass weight of 21.2 kg for Merino lambs fattened over 63 days. The lower carcass weights possibly due to the poorer feedlot performance of Merino lambs compared with South African Mutton Merinos. Sheridan *et al.* (2003) reported a cold carcass weight of 24.6 kg over a 56day feedlot period where South African Mutton Merinos lambs received ahigh energy diet (12.7 MJ/kg).

The carcass weights of the 0% and 0.42% urea treatments were significantly higher ($P < 0.05$) than the cold carcass weights of 0.83%, 1.25%, and 1.66% urea treatments. The higher carcass weights obtained from the 0% and 0.42% urea treatments are not in agreement with results reported by Zinn *et al.* (2000). They reported similar carcass weights for steers fed a diet containing either 20% NPN or 40% NPN. In that trial, the steers receiving the diet with 40% NPN were 50 days longerin the feedlot, which can explain why there were no differences observed in cold carcass weight. There tended ($P < 0.10$) to be a linear relationship between the level of urea supplementation and cold carcass weight as seen in Figure 3.11.

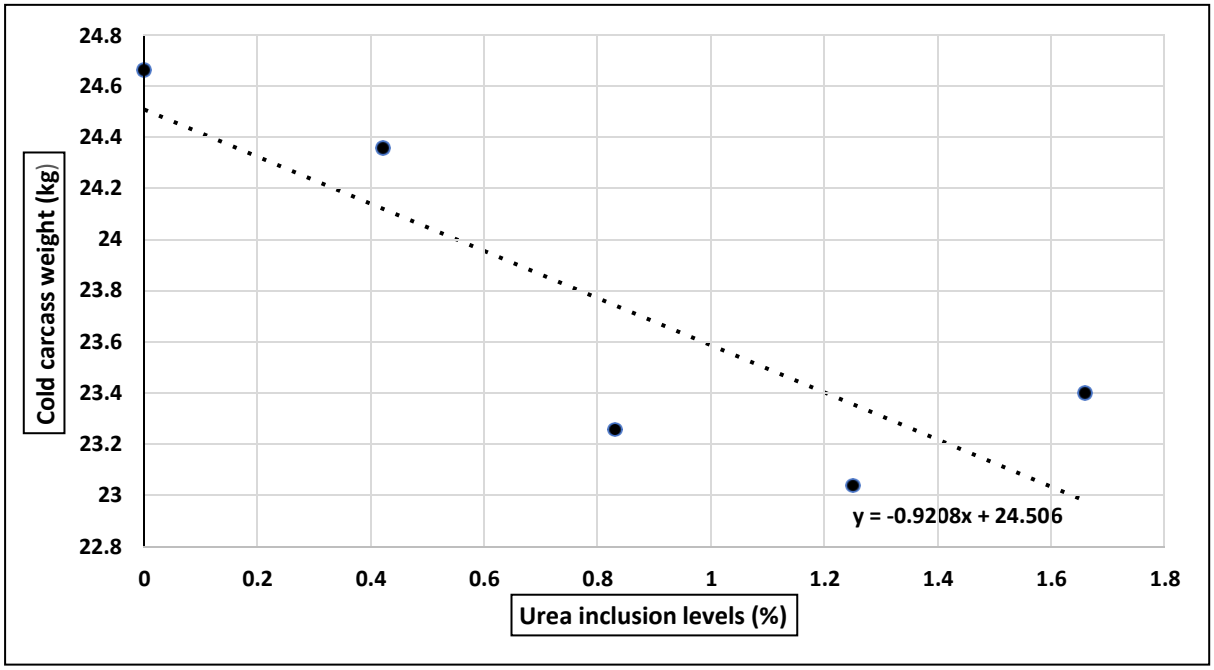

Figure 3.11 The linear relationship (*P* <0.10) between urea inclusion levels and cold carcass weight (kg).

Dressing percentage

The calculated dressing percentage of each treatment is shown in Table 3.8 above. The dressing percentage was calculated with cold carcass weights as a percentage of the final body weight of lambs. The mean dressing percentage for this trial was 47.89%, with values ranging from 48.57% to 47.40%. Brand *et al.* (2017) reported a dressing percentage of 48.9% and 49.4% for two groups of South African Mutton Merino lambs finished over 63 days. Another study reported a 50.1% dressing percentage for South African Mutton Merino lambs fed for 56 days on a high-energy diet (Sheridan *et al.,* 2003).

The 0.42% urea treatment tended (*P* <0.10) to have a higher dressing percentage than the 0.83% and 1.66% urea treatments. Even though there were differences in the cold carcass weights between treatment diets, there were no significant differences in dressing percentage. Muir *et al.* (2008) did a review on the dressing percentage of lambs in New Zealand, in which they concluded that a higher live weight does not result in a higher dressing percentage, but rather a high rate of growth and fat deposition. In this trial, lambs were fattened the same number of days, with iso-energetic diets, which may explain why there are no significant differences obtained.

4. Conclusions

When the results for treatments 1 to 4 (0% urea to 1.25% urea) are compared, then there is a clear indication that weight gain decreased, ADG decreased and FCR were poorer as the level of urea increased. The treatment with 1.66% urea, however, did not follow the same trend and did not differ from the 0% urea treatment in terms of weight gain, ADG, and FCR. When considering all treatments, results suggest that urea can be included up to 1.66% in lamb feedlot diets when replacing CSOCM. Results furthermore suggest that protein quality might be more important in the early feedingphase (day 1-21) when compared with the full feedlot feeding period. Further studies are needed to determine the breakpoint in days on feed where protein quality does notimpact growth performance anymore. DMI for this trial followed the trend as expected, with increasing levels of urea resulting in lower intake values. DMI values of this trial 1.698 kg/lamb/day is representative of DMI values achieved by South African Mutton Merino lambs in feedlots. The DMI for 0% and 0.42% urea differed (P <0.05) from the DMI of lambs receiving the 0.83%, 1.25% and 1.66% urea treatments.

There was a difference (P <0.05) between the cold carcass weights of treatments. The lambs receiving the 0% and 0.42% urea treatments had higher cold carcass weights. There was a tendency (P <0.10) for the dressing percentage of the 0.42% urea treatments being higher than the dressing percentage of the 0.83% and 1.66% urea treatments.

Chapter 4

The effect of protein quality on rumen fermentation dynamics of sheep

1. Introduction

To support or better explain the growth performance data, a rumen fermentation study was conducted, concurrent with the growth performance study. The study comprised of an apparent digestibility study as well as measurements of the rumen parameters, pH, and rumen NH_3-N. The material and methods, results, discussion, and conclusion are discussed below.

2. Materials and methods

2.1 Experimental design, animals and treatments

Five rumen cannulated Merino wethers, approximately 4-5 years of age with a body weight of 81.8 kg (±3.1 kg) were used in a 5x5 Latin square design to investigate the effect of incremental levels of urea supplementation on specific rumen fermentation parameters. Before the start of the trial, all the wethers were taken to the crush, where they were treated for internal parasites using Zolvix® sheep oral solution, injected with a Multimin®(Virbac) subcutaneously and then they were weighed individually.

After a preliminary adaptation period of 14 days, wethers entered an 81-day trial, which consisted of five experimental periods. Each period was 16 days, each consisting of 10 days for adaptation to the new experimental diet and six days of sampling and data collection.

The experimental treatments were arrayed in a 5x5 Latin Square design arrangement, with each of the treatments appearing once in each experimental period (row) and once for each animal (column). The experimental treatments were similar to the treatments used in the growth trial

The random allocation of experimental diets to the animals according to a 5x5 Latin Square design is shown in Table 4.1. Experimental animals were identified by their ear tag numbers.

Table 4.1 The random allocation of experimental diets to animals.

	Animal[1]				
Experimental period and treatments[2]	**P1312**	**P1309**	**P1303**	**P1311**	**P1302**
1	A	B	C	D	E
2	C	D	E	B	A
3	B	C	A	E	D
4	E	A	D	C	B
5	D	E	B	A	C

[1]Ear tag number of the five rumen cannulated experimental wethers
[2]Each experimental period consisted of 16 days (10 day's adaptation and 6 day's collection)
A = 0.00% urea (Treatment 1)
B = 0.42% urea (Treatment 2)
C = 0.83% urea (Treatment 3)
D = 1.25% urea (Treatment 4)
E = 1.66% urea (Treatment 5)

2.2 Digestibility study

A total fecal collection study was performed to estimate the apparent nutrient digestibility of the experimental diets. Wethers were allowed a smooth adaptation to each experimental diet over a 10day period. From days 1 - 7 of each adaptation period wethers were housed outside in individual pens that were directly next to each other, which allowed animals to socialize. The pen sizes were 3.5 m x 2 m with sloped concrete floors, feed troughs, and self-filling water buckets. On the morning of day 8, wethers were placed in their respective metabolic crates next to each other, fecal bags was fitted, and they were allowed to adapt for 3 days.

On day 11 of each experimental period, the sample collection period began. Thisconsisted of six days in which data and samples of feed, orts, feces, and rumen fluid were collected as discussed in section 2.3 below.

After six days of collection, wethers were taken out of metabolic crates and allowed to walk freely and exercise for 3 – 4 hours before they were placed in their individual pens with their new allocated experimental diet. Animal weights were recorded before and after each experimental period to monitor weight and health status. After each experimental period, each wether's cannulae was cleaned and disinfected, and the surrounding wool was sheared. Water buckets were cleaned daily and fresh water was available *ad libitum* for the duration of the trial. Animals were fed

twice daily at 08h00 and 16h00 for both the adaptation and sampling period to stimulate feed intake whilst offering fresh pellets at all times.

2.2.1 Data and sample collection

The trial consisted of five sample collection periods in which data collection and sampling procedures remained consistent throughout.

Feed and orts

Feed was weighed and the amount offered was recorded for the duration of the trial. Animals were fed twice a day at 08h00 and 16h00. During the collection period, for six days, orts were weighed in the morning before feeding and the weight was recorded. Buckets were emptied when orts were removed, to calculate digestibility as accurately as possible.

No feed or orts samples were taken during this experiment.

Fecal collections

After orts collection and before feeding took place, fecal bags were emptied and the weight of the feces was recorded. Bags were completely emptied and sealed, to prevent any loss or contamination, which would have affected digestibility results. The feces were properly mixed and a 10% representative sample was taken, which was placed in an air-tight sealed plastic bag and stored in a freezer at -20^{o}C. The remaining feces were discarded. The faecal samples were pooled for each animal per experimental period (Köster *et al*., 1996; Olson *et al*., 1999; Mentz *et* al., 2015).

2.2.2 Parameters measured

The following parameters were monitored and calculated using the data collected during the five sample collection periods and the results of the laboratory analysis.

Feed intake and digestibility

- Dry matter intake/day
- Apparent total tract digestibility for:
    1. Dry matter

2. Organic matter
3. Crude protein
4. Neutral detergent fibre
5. Starch
6. Energy

The following equation was used to determine the digestibility coefficient as described by McDonald *et al.* (2011):

$$\text{Digestibility coefficient} = \frac{\text{nutrient consumed} - \text{nutrient in feces}}{\text{nutrient consumed}} \times 100$$

2.3 Rumen fermentation study

Rumen fluid was collected to analyze the effect of different levels of urea in the diet on specific rumen fermentation parameters.

2.3.1 Data and sample collection

Rumen fluid was collected from four predetermined areas (top left and center and bottom left and center) to obtain a representative sample of the entire rumen (Mynhardt *et al.,*2016). Rumen fluid was collected twice a day from day two to five of each collection period. Rumen fluid was collected at 12hour intervals, with the sampling time, shifting three hours every day, to mimic a 24hour period and account for the diurnal variation (Mentz *et al.,*2015).

Directly after rumen fluid was collected the pH was measured with a portable pH meter (which was calibrated before each collection using the buffer solutions of pH 4 and pH 7) and then the data were recorded. For NH_3-N analysis, samples were preserved by adding 5 mL of 50% H_2SO_4 solution per 30 mL of rumen fluid (Broderick & Kang, 1980). The labeled sample bottles were then stored at -20^{o}C for later analysis. At the time of laboratory analysis, the NH_3-N samples were pooled as follows:

- 00h00 + 03h00
- 06h00 + 09h00
- 12h00 + 15h00
- 18h00 + 21h00

Table 4.2 Rumen sampling schedule during each collection period.

		Time of rumen fluid collection	
Collection period	**Day of collection period**	**Morning**	**Afternoon**
1-5	2	06h00	18h00
1-5	3	03h00	15h00
1-5	4	00h00	12h00
1-5	5	09h00	21h00

2.3.2 Parameters measured

The following rumen fermentation parameters were derived from rumen fluid collection and analysis.

- pH
    - Diurnal, minimum, maximum, and average rumen pH
    - Time (minutes) that pH was under 5.5 (sub-clinical acidosis) (Hibbard *et al*., 1995)

The area under the curve (AUC) was determined from the data obtained during the *in vivo* sampling periods where a portable pH meter was used to measure the pH of the rumen fluid samples. Data was converted into AUC for a 24hour period, using an approximation method (where time intervals are separated into rectangular areas) using the formula for area from Microsoft Excel (Microsoft Office, 2016).

Area = Base x Height (i.e. Area = time interval x pH measurement)

- NH_3-N production
    - Diurnal, minimum, maximum, and average NH_3-N production

2.4 Statistical analysis

The data were analyzed statistically as a 5 x 5 Latin Square design with the GLM model (Statistical Analysis System, 2019). Repeated Measures Analysis of Variance with the GLM model was used for repeated period measures. Means and standard error were calculated and the significance of difference (P <0.05) between means was determined by Fischer's test (Samuels, 1989).

The linear model used is described by the following equation:

$$y_{ijk} = \mu + p_i + S_j + T_k + e_{ijk}$$

Where: i = 1,2,3,4,5 j= 1,2,3,4,5 k=1,2,3,4,5

y_{ijk} = the observation on the experimental unit in the i^{th} row and the j^{th} column

μ = overall mean

p_i = effect of the i^{th} period (row effect)

y_j = the animal effect (column effect)

T_k = the effect of the k^{th} treatment

e_{ijk} = the random, independent experimental errors with the mean 0 and variance σ^2

3. Results and discussion

3.1 The effect of different dietary levels of urea on feed intake and apparent total tract digestibility

The DMI and apparent digestibility coefficients are shown in Table 4.3. The DMI did not differ (P >0.05) between treatments, therefore, the feeding level was not a factor that could have influenced the apparent digestibility of the diets. Apart from starch digestibility, there were differences (P <0.05) in the apparent total tract digestibility coefficients for DM, OM, CP, NDF, and energy.

Table 4.3 The effect of different inclusion levels of urea on mean (±SE) feed intake and apparent total tract digestibility in sheep.

	Treatment					
Parameters	**0% urea**	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**	**±SE[1]**
Dry matter intake (g/day)	986	1031	1235	1061	1041	153
Apparent digestibility coefficients :						
Dry matter (%)	67.29^{c}	70.12^{bc}	71.22^{bc}	74.57^{ab}	77.83^{a}	2.262
Organic matter (%)	71.85^{c}	72.85^{c}	74.90^{bc}	78.90^{ab}	81.22^{a}	1.912
Crude protein (%)	70.28^{c}	73.20^{bc}	78.19^{ab}	80.20^{a}	81.56^{a}	2.354
Neutral detergent fibre (%)	49.95^{a}	46.42^{ab}	45.70^{ab}	40.30^{b}	41.47^{ab}	3.490
Starch (%)	97.68	98.03	98.16	97.57	98.02	0.425
Energy (%)	71.04^{c}	74.37^{bc}	74.86^{bc}	78.19^{ab}	80.86^{a}	2.128

^{a,b,c} Row means with a different superscript differ significantly (P <0.05)

^{d,e} Row means with a different superscript tend to differ (P <0.10)

[1]±SE: Standard error

The apparent digestibility coefficient of starch ranged from 97.57% to 98.16%, which is in agreement with other studies. Lee-Rangel *et al.* (2012) conducted a digestibility study in which sheep received a high concentrate diet with either a ruminal buffer or exogenous amylolytic enzymes. The non-significant effect of the exogenous amylolytic enzymes on starch digestibility was mainly due to the high rumen digestibility of starch in that trial. Total tract starch digestibility did not differ ($P > 0.05$) with values ranging from 96.2% to 97.4%. In a summary of digestibility studies with beef feedlot cattle, the total tract digestibility of maize starch ranged from 89.3% to 99.2% (Owens & Zinn, 2005). Digestibility coefficients greater than 98% weretypically observed in cattle receiving diets with high ruminal digestibility of starch as a result of grain processing.

The experimental diets for this trial were in pelleted form, thus the gelatinization (disruption of starch granules through heat during the pelleting process) was probably the reason for the high digestibility of starch (Lee-Rangel *et al.,* 2012; Owens & Basalan, 2016). The results from the meta-analysis by Owens & Zinn (2005) indicated that total tract digestibility of starch was maximized when diets were rich in N but low in NDF. Because the experimental diets were formulated on an iso-nitrogenous and iso-fibrous basis, it can explain why there were no differences observed in the total tract digestibility of starch. Although the level of starch in the diets was increased as urea levels increased, it had no effect on the extent of total tract starch digestibility.

Increasing levels of concentrate in the diets of ruminants usually result in increased total tract digestibility of DM and OM (Ramos *et al.,* 2009). However, fiber digestibility can decrease especially if ruminants are fed grain-based concentrates (Carro *et al.,* 2000). The apparent total tract digestibility of NDF ranged from 40.30% to 49.95%. This is in agreement with results obtained from several other ruminant digestibility studies (Simpson *et al.,* 2000; Smith, 2008; Mynhardt *et al.,* 2016). The 0% urea treatment had a higher ($P < 0.05$) NDF digestibility than the 1.25% urea treatment. This was not unexpected, as the level of starch in the diet increased as urea levels increased, although there was no difference between the 0% and 1.66% urea treatment. A meta-analysis done by Ferraretto *et al.* (2013) highlighted the effect of dietary starch concentration on *in vivo* NDF digestibility of dairy cows. The digestibility of dietary NDF decreased 0.61%-units ruminally and 0.48%-units total track with a 1%-unit increase in dietary starch content.

The negative effect of higher starch levels on NDF degradation is thought to be the result of (1) preference of ruminal microorganisms for starch rather than NDF; (2) decreased ruminal pH caused by rapid degradation of starch and (3) preferential proliferation of starch digesting bacteria caused by competition for essential nutrients. One explanation as to why there was a difference in NDF digestibility between 0% and 1.25% urea, but not 1.66% urea, can be explained by the second point above. The

pH of 0% urea was higher (P <0.05) than the 1.25% urea treatments, but it was not significantly higher than the 1.66% urea treatments.

Although diets were formulated to have the same NDF concentration, the proportion of cellulose, hemicellulose and lignin within NDF will vary greatly among feeds, and consequently, the digestibility of the NDF fraction is variable. The 0% urea treatment had a greater proportion of the dietary NDF contributed by CSOCM compared with the 1.66% urea treatment that had almost no dietary NDF supplied from CSOCM. Esminger *et al.* (1990) reported that bagasse is high in fiber, with low DMD (about 25%). Preston (1995) reported 30% digestibility for sugarcane bagasse. The 1.66% urea treatment had a larger proportion of indigestible NDF as a result of the higher inclusion levels of sugarcane bagasse. The NDF digestibility of CSOCM is higher than the NDF digestibility of sugarcane bagasse, as a result of lower lignin concentrations in CSOCM. The crude fiber content of sugarcane bagasse is composed of approximately 50% cellulose, 25% hemicellulose, and 25% lignin (Xu *et al*., 2011; Huang *et al*., 2012).

The apparent total tract digestibility of CP for this trial ranged from 70.28% to 81.56%. The digestibility increased as the levels of urea inclusion were increased in the experimental diets. The 1.66%, 1.25% and 0.83% urea treatments had higher (P <0.05)apparent total tract CP digestibility than the 0% urea treatment, and the 1.66% and 1.25% urea treatments were also higher (P <0.05) than the 0.42% urea treatment. Xu *et al.* (2019) conducted an experiment in which they investigated the effects of partially replacing soybean meal with incremental levels of urea on nutrient digestion. In that study, urea was increased from 0% up to 3% of diet DM, but the other feed ingredients remained similar between treatment diets, thus treatment diets were not formulated on an iso-nitrogenous basis. They observed a linear increase (P <0.01) in the apparent digestibility of CP as the urea levels were increased. The metabolic fecal N was constant between treatment diets, but according to the calculation formula for CP apparent digestibility, the CP apparent digestibility is positively correlated with dietary CP level when the metabolic fecal N is constant (Waldo, 1968).

Khattab *et al.* (2013) conducted a digestibility study in which lambs received a high concentrate diet with urea inclusions ranging from 0 to 1.5%. In that trial urea was included at the expense of soybean meal with CP apparent digestibility ranging from 72.3% to 74.5%. There was a linear increase (P <0.05) in DM, OM, and CP digestibility as urea levels were increased. A similar increase in CP digestibility with incremental levels of urea had been obtained by Pingel & Trenkle (2006). In a study by Wanapat *et al.* (2013) where the level of CSOCM was increased from 10.9% to 32.8% at the expense of urea, there was a decrease in apparent total tract CP digestibility from 65.9% to 61.6%. Giri *et al.* (2000) also obtained significantly higher (P <0.05) CP digestibility with diets containing urea as opposed to other N sources. This is in contrast with the results obtained by de Jesus *et al.* (2012), in which dairy cows had a higher apparent CP digestibility for diets containing soybean meal compared with

urea. In the studies mentioned above where the CP apparent digestibility was higher for treatment diets with higher urea inclusion levels, the urea was included at the expense of a plant protein oilcake. Most oil seeds are subjected to a heating process to produce the oil-cake by-product. As the temperature and time of heating increase, the amount of acid detergent insoluble nitrogen (ADIN) in the feed ingredient also increases (McNiven *et al.,* 1994; Schroeder *et al.,* 1995). The experimental diets used in this trial were in pelleted form, thus the CSOCM went through two heating processes.This might have increased the levels of ADIN up to levels that could have had a negative correlation with CP apparent digestibility, thus as the levels of CSOCM were increased in the experimental diets, the CP apparent digestibility decreased.

The energy apparent digestibility of the 1.66% urea treatment was higher (P <0.05) than the energy apparent digestibility of the 0%, 0.42% and 0.83% urea treatments, while the 1.25% urea treatment was also higher (P <0.05) than the 0% urea treatment. There is an increase in energy apparent digestibility as the levels of urea were increased. Due to the high digestibility of starch for this trial (more than 97%), it would be expected that energy apparent digestibility will increase with an increased proportion of energy intake in the form of starch. As the levels of urea were increased, there was a concurrent increase in the amount of maize in the experimental diets, therefore more energy in the form of starch. This is in agreement with other studies in which energy apparent digestibility was increased as the level of concentrate in the diet increased (Montgomery & Baumgardt, 1965)

Although the overall apparent total tract digestibility was improved as the levels of urea were gradually increased in the experimental diets of cannulated wethers, thiswas not evident from the performance of the lambs in the feedlot trial.

3.2 The effect of different dietary levels of urea on rumen fermentation dynamics

Table 4.4 The effect of different inclusion levels of urea on mean, minimum and maximum (±SE) rumen fermentation parameters in sheep and time spent below pH 5.5 during a 24hour period.

	Treatment[1]					
Parameters	**0% urea**	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**	**±SE[1]**
Rumen pH						
Average rumen pH	5.72^{a}	5.51^{ab}	5.45^{ab}	5.35^{b}	5.47^{ab}	0.110
Minimum pH	5.40^{a}	5.22^{ab}	5.27^{ab}	5.02^{b}	5.17^{ab}	0.090
Maximum pH	6.14^{a}	5.72^{ab}	5.65^{b}	5.69^{b}	5.65^{b}	0.138
[2]Minutes < pH 5.5	396^{a}	756^{ab}	792^{ab}	1008^{b}	828^{ab}	164.9
Rumen NH_3-N[3]						
Average NH_3-N	13.09^{b}	16.62^{ab}	21.04^{a}	20.06^{a}	20.62^{a}	2.473
Minimum NH_3-N	10.52^{b}	16.04^{ab}	19.64^{a}	15.01^{ab}	17.67^{ab}	2.354
Maximum NH_3-N	15.05^{b}	16.92^{ab}	23.31^{a}	24.33^{a}	23.30^{a}	3.693

^{a,b,c,} Row means with the same superscript do not differ significantly (P >0.05)

^{d,e} Row means with a different superscript have a tendency (P <0.10)

[1]±SE: Standard error

[2]Minutes < pH 5.5: Time spent below pH 5.5, calculated from the area under the graph

[3]NH_3-N (mg NH_3-N/100ml)

Table 4.5 The linear and quadratic relationships between the different urea inclusion levels and the dependent variables.

Variable	Linear relationship	*P*-value	Quadratic relationship	*P*-value
Average rumen pH	y = -0.16x + 5.63	0.130	y = 0.26x^2- 0.58x + 5.72	**0.045**
Minimum pH	y = -0.25x + 5.38	0.185	y = -0,001x^2 - 0,24x + 5,38	0.509
Maximum pH	y = -0.20x + 6.13	0.176	y = 0.38x^2 - 0.83x + 6.25	**0.038**
Minutes < pH 5.5	y = 269x + 531	0.110	y = -371x^2 + 887x + 403	0.085
Average NH_3-N	y = 4.46x + 14.58	0.060	y = -4.69x^2 + 12.24x + 12.96	0.057
Minimum NH_3-N	y = 2.21x + 9.91	0.311	y = -2.03x^2 + 5.59x + 9.21	0.603
Maximum NH_3-N	y = 6.14x + 20.49	0.101	y = -4.59x^2+ 13.39x + 14.21	**0.008**

Rumen pH

Table 4.6 The effect of different inclusion levels of urea on rumen pH at different hours of the day.

	Treatment					
	0% urea	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**	**±SE[1]**
Rumen pH						
pH 00h00	5.62^{d}	5.38^{de}	5.31^{de}	5.25^{e}	5.46^{de}	0.136
pH 03h00	5.85^{d}	5.72^{de}	5.43^{de}	5.50^{e}	5.36^{de}	0.169
pH 06h00	6.14^{a}	5.62^{b}	5.58^{b}	5.69^{ab}	5.65^{b}	0.151
pH 09h00	5.96	5.62	5.65	5.61	5.63	0.198
pH 12h00	5.40^{ab}	5.22^{abc}	5.47^{a}	5.02^{c}	5.17^{bc}	0.087
pH 15h00	5.63	5.60	5.55	5.39	5.65	0.123
pH 18h00	5.52	5.43	5.27	5.19	5.45	0.199
pH 21h00	5.63^{d}	5.46^{de}	5.31^{de}	5.18^{e}	5.41^{d}	0.167

^{a,b,c} Row means with different superscript differ significantly (P <0.05)

^{d,e} Row means with a different superscript tend to differ (P <0.10)

^{1}±SE: Standard error

There was a significant difference (P <0.05) in average pH between the 0% urea treatment and the 1.25% urea treatment, with the 0% urea treatment being the highest and the 1.25% urea treatment the lowest. The pH values ranged from 5.35 to 5.72. There was a quadratic relationship (P <0.05) between urea inclusion levels and the average rumen pH. The pH values for this trial were in general lower than other ruminant studies (Smith, 2008; Lee-Rangel *et al.,* 2012; Mentz *et al.,* 2015). In these trials sheep generally received diets with higher fiber content. The low rumen pH is in agreement with other studies in which animals received high concentrate diets in pelleted form. Ørskov *et al.* (1974) obtained a rumen pH of 5.2 in lambs receiving a ground pelleted maize diet, compared with a rumen pH of 6.1 for the same diet fed as whole loose maize. The lack of fibrous structure in the finely ground and pelleted diets results in a lack of tactile stimuli, inadequate abrasion of rumen epithelium, and reduced ruminal motility.

The minimum and maximum pH for all unpooled data points are depicted in Table 4.4. The 1.25% urea treatment had a lower minimum pH (P <0.05) than the 0% urea treatment. The 0% urea treatment on the other hand had a significantly higher (P <0.05) maximum pH than the 0.83%, 1.25%, and 1.66% urea treatment. A quadratic relationship (P <0.05) was observed between urea inclusion levels and maximum pH. As the urea levels increased in experimental diets, the inclusion of maize meal was also

increased. Thus it was expected that pH will decline as urea levels were increased, and therefore these minimum and maximum pH values are as expected. The higher maize levels had a more dominant effect compared with the alkalizing effect of the more urea.

Figure 4.2 shows that all treatments followed a similar trend in rumen pH for the pooled time points over a 24hour period. Differences in pH measurements were observed at 06h00 and 12h00, with the 0% urea treatment being higher (P <0.05) than the 0.42%, 0.83% and 1.66% urea treatments at 06h00. At 12h00 the 0.83% treatment was higher (P <0.05) than the 1.25% and 1.66% urea treatments, and the 0% treatment was also higher (P <0.05) than the 1.25% urea treatment. Rumen pH was at its lowest for all treatments at 12h00, except for the 0.83% urea treatment. These low pH measurements were 3 hours after the morning feeding. Another drop in rumen pH was observed at 18h00, which was 3 hours post afternoon feeding. This was expected as it can take up to 5 hours for starch to completely disappear from ground maize (Sinclair *et al*., 1993). Rumen pH varies throughout the day but is at its lowest 2-6 hours after morning feeding (Millen *et al.,* 2016). High RAN concentration will increase rumen pH, but rumen fluid samples were taken three hours post-feeding. Thus RAN levels were not high enough at that time to neutralize the acidic rumen fluid (Sinclair *etal.,* 1995).

Acidosis, characterized by low ruminal pH (Owens *et al.,* 1998) is thought to be a prevalent digestive disorder in feedlot animals fed high-grain diets. It is commonly classified as either subacute (subclinical) or acute (clinical or lactic) acidosis based on the severity of rumen pH depression, time that rumen pH remains depressed, and whether lactate accumulates in rumen fluid. A rumen pH of 5.2 is considered the threshold between the clinic and subclinical acidosis. Great ruminal pH fluctuations are always associated with clinical and subclinical acidosis. Figure 4.2 shows the ruminal pH fluctuations over a 24hour period. A rumen fluid pH of 5.6 is considered the threshold between subclinical acidosis and a healthy rumen (Owens *et al.,* 1998; Galyean & Rivera, 2003). Subclinical acidosis is generally characterized by ruminal pH between 5.6 and 5.2. It is postulated by some authors (Owens *et al.,* 1998) that subclinical and clinical acidosis is rather a response to the time spent below a pH threshold rather than the lowest pH point *per se.*

The time that pH was below 5.5, which was considered sub-clinical acidosis (Hibbard *et al.,* 1995), is shown in Table 4.4. The 0% urea treatment spent significantly less time (P <0.05) below pH 5.5 compared with the 1.25% urea treatment. Figure 4.3 indicates that during this trial only the 0% urea treatment had spent more time above pH 5.5 than below pH 5.5. In general, the time spent below pH 5.5 in this trial was more than data from other ruminant studies. Although the time spent below pH 5.5 had to be determined from the area under the graph, it is still evident that the wethers had sub-clinical acidosis during this trial. A reduction

in DMI occurs concomitantly with low rumen pH, thus an animal with subclinical acidosis will have fluctuating DMI for days. Although daily DMI is presented as an average for the different experimental periods, it was evident during the trial that the 1.25% urea treatment had the least consistent day-to-day DMI, which could be explained by the low rumen pH, and amount of time spent below pH 5.5. Schwartzkopf-Genswein *et al.* (2004) observed that cattle with fluctuating intakes spent more time at ruminal pH below 5.5, and this severely inhibit fiber digestion (Yang 2002). This explains why the 1.25% urea treatment had the lowest NDF digestion for this trial.

From Figure 4.6 it is clear that the wethers used in this trial had damaged rumen walls. One of the most important reasons for the appearance of ruminal acidosis is a decrease in the absorptive capacity of the rumen which is thus unable to maintain a stable pH (Hernández *et al.,* 2014). The VFA in the rumen contacts with the chemical receptors of the epithelium and this then reduces the ruminal motility which assists with VFA absorption. When the rumen epithelium is damaged this feedback signal to the brain is reduced, and thereby reducing the absorption of VFA from the rumen. From Figure 4.6 it is evident that there are very few ruminal papillae, and thus a smaller surface area, which reduces the absorption capacity of the rumen wall. Structural changes of the epithelium increase the animal's susceptibility to microbial infection and alter rumen metabolism and nutrient absorption (Steele *et al.,* 2009). Thus the damage caused to the rumen wall may be another reason for the low rumen pH measurements obtained in this trial.

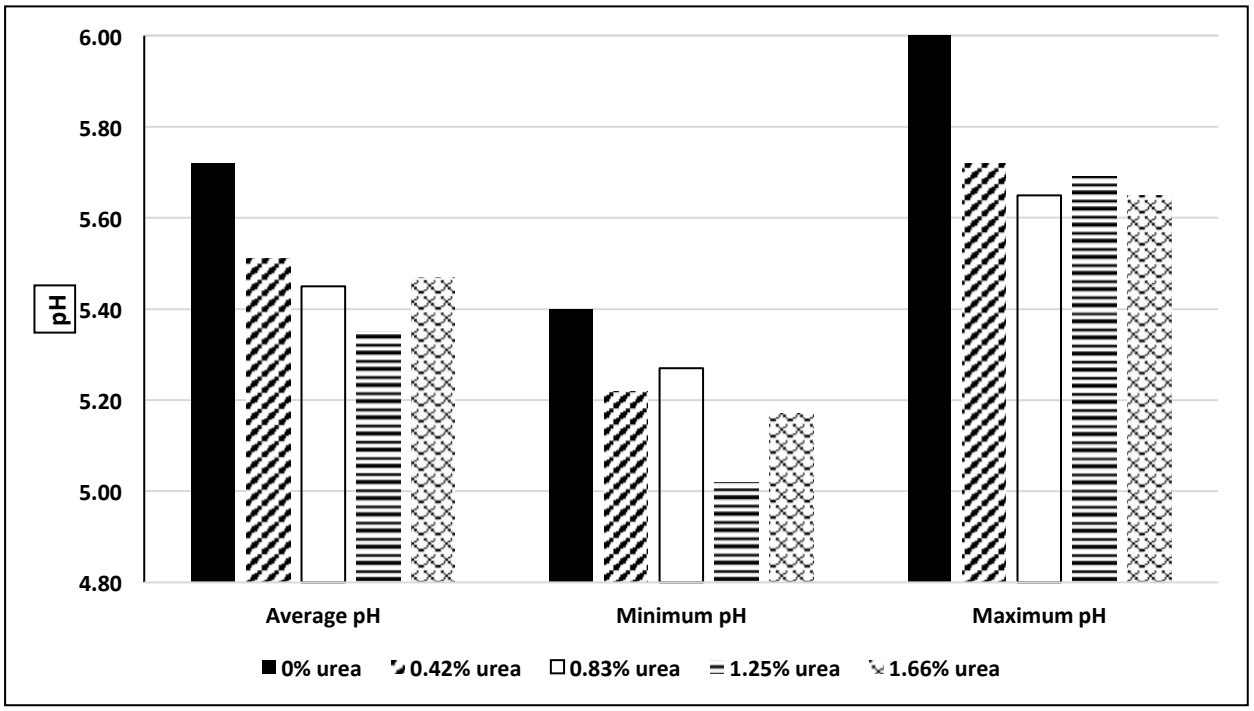

Figure 4.1 The effect of incremental levels of urea supplementation on rumen pH.

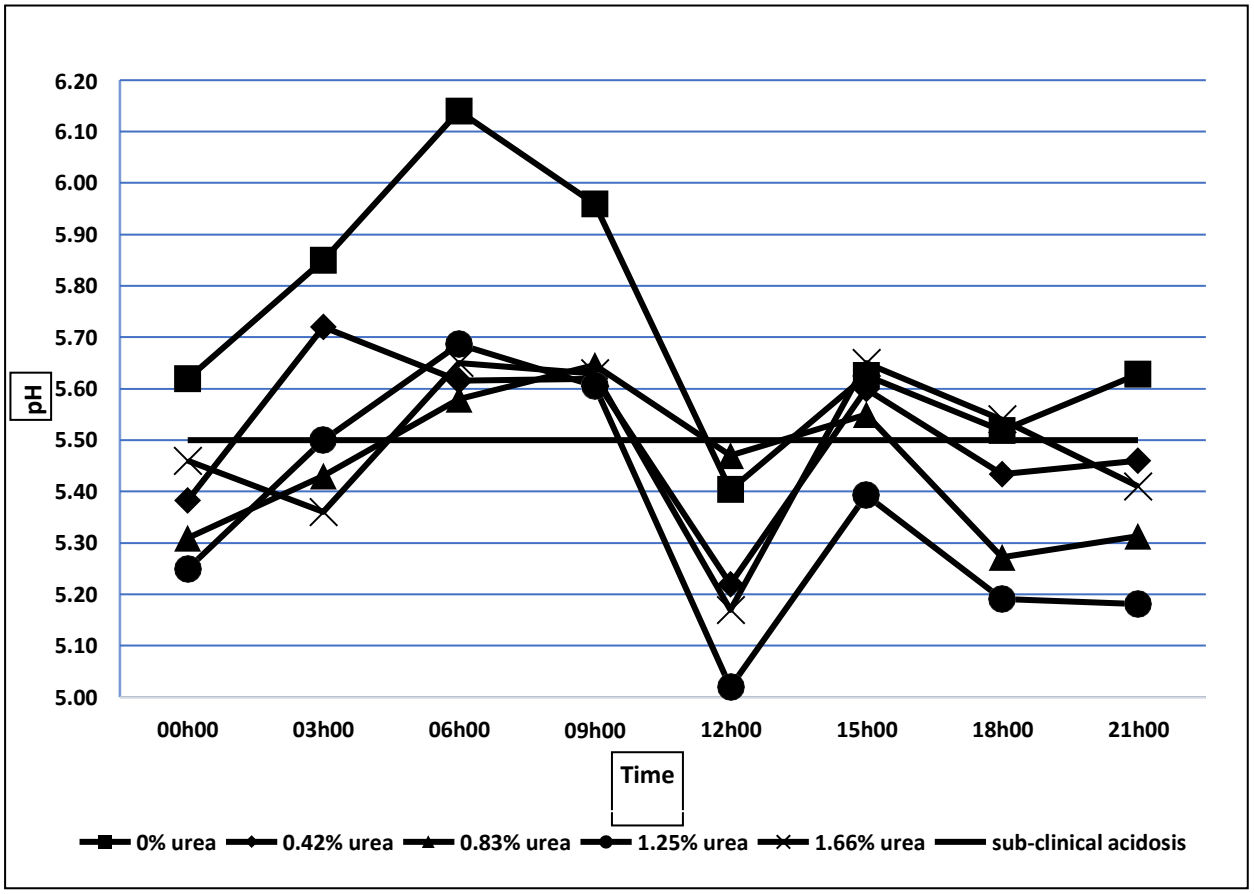

Figure 4.2 The rumen pH of sheep (over a 24hour period) supplemented with different levels of urea.

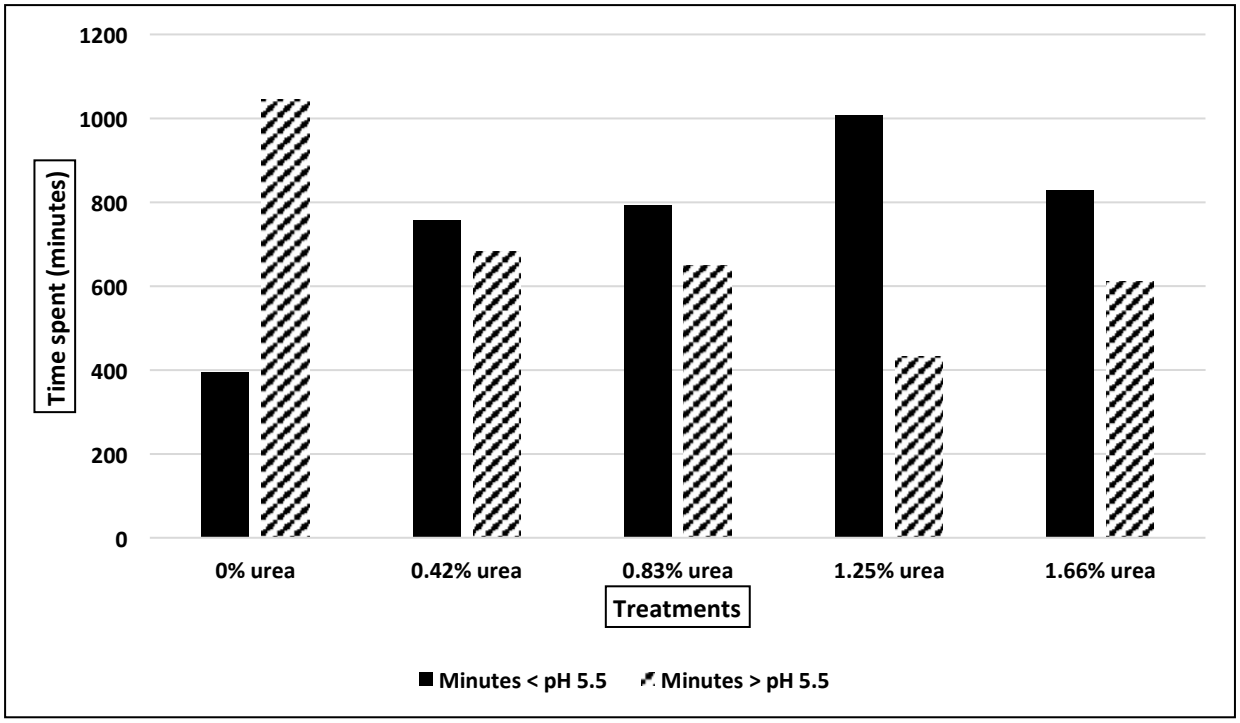

Figure 4.3 The effect of incremental levels of urea supplementation on the time spent above or below pH 5.5 during 24 hours.

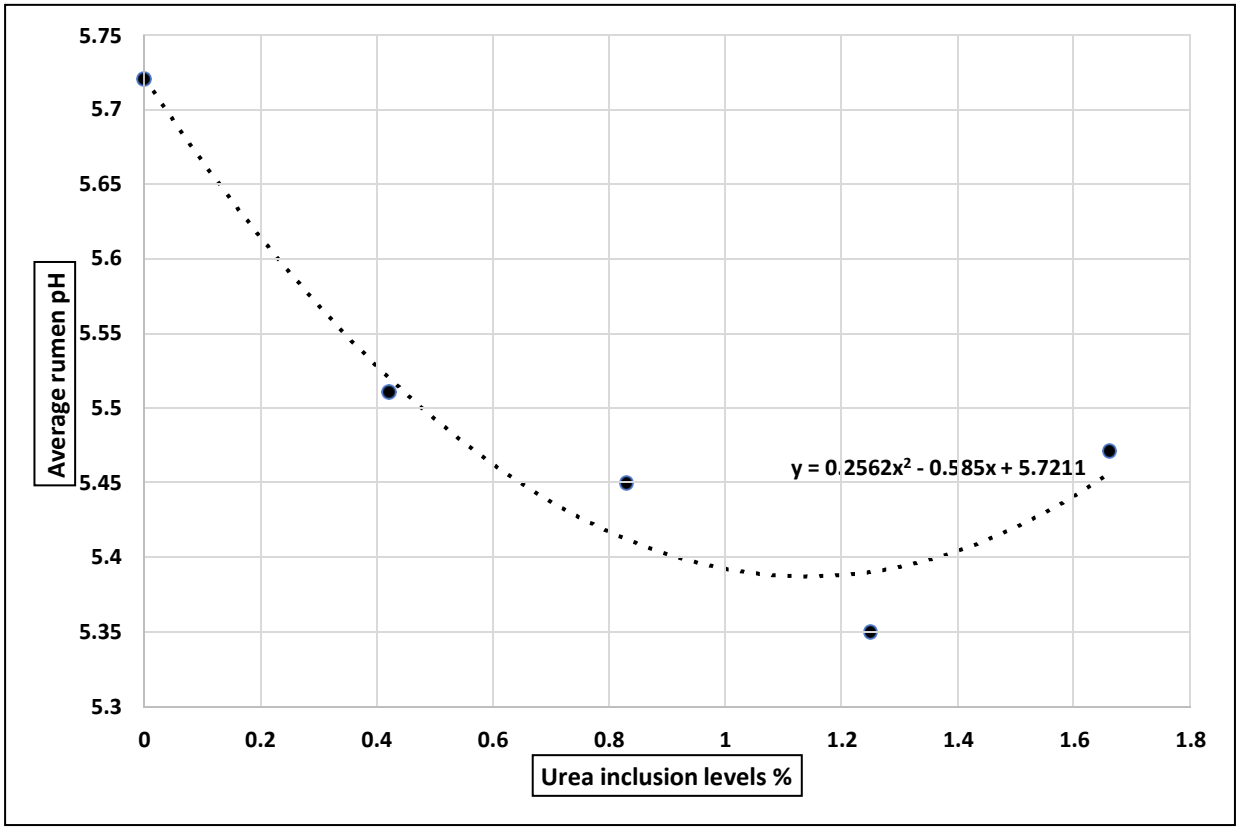

Figure 4.4 The quadratic relationship (*P* <0.05) between urea inclusion levels and average rumen pH.

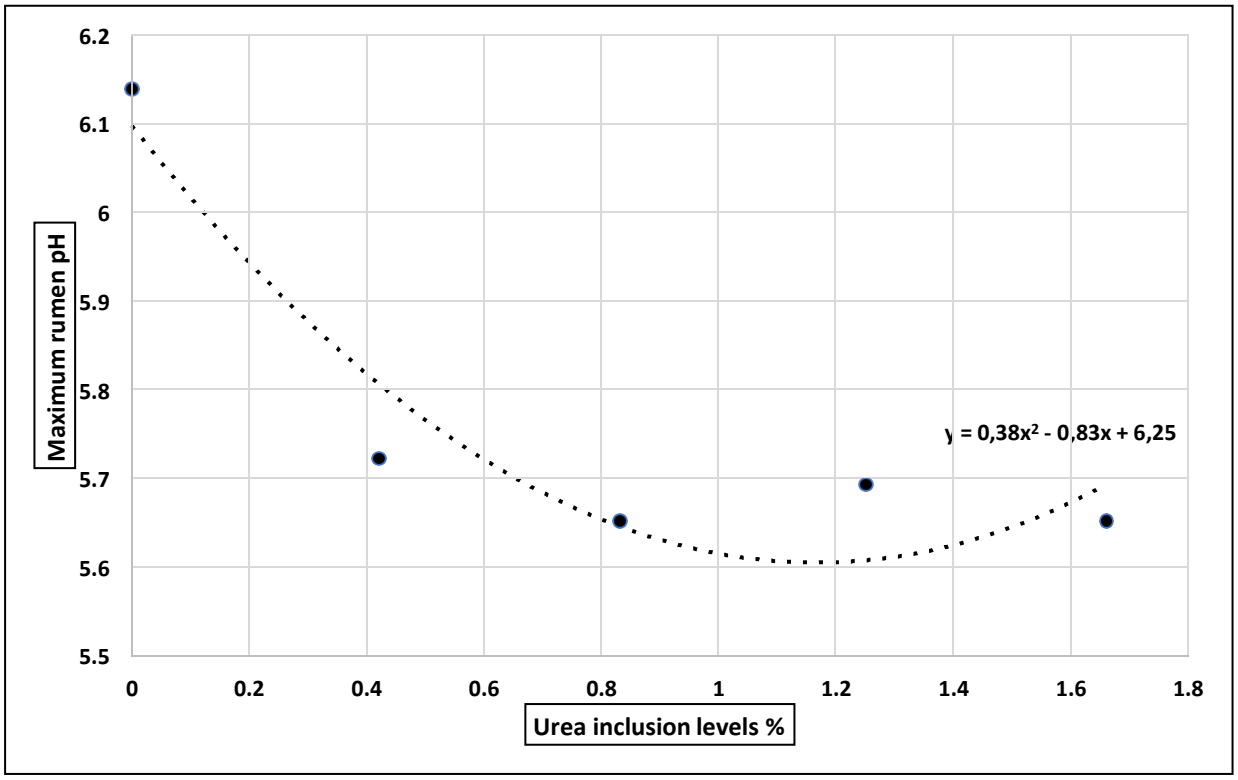

Figure 4.5 The quadratic relationship (*P* <0.05) between urea inclusion levels and maximum rumen pH.

Figure 4.6 The damaged rumen wall of one of the wethers used in this trial.

Adapted from Grimsell, A. 2019. MSc (Agric) Animal Science: Nutrition Science, dissertation, Dept. of Animal and Wildlife Sciences, University of Pretoria, South Africa.

Rumen ammonia nitrogen

Table 4.7 The effect of different inclusion levels of urea on rumen NH_3-N concentrations at different hours of the day.

	Treatment					
	0% urea	**0.42% urea**	**0.83% urea**	**1.25% urea**	**1.66% urea**	**±SE[1]**
Rumen NH_3-N[2]						
00h00	12.73	16.71	19.65	19.16	20.24	4.431
06h00	10.52^{d}	16.04^{cd}	19.64^{d}	15.01^{cd}	17.67^{cd}	4.585
12h00	15.05^{d}	16.81^{cd}	21.57^{c}	21.73^{c}	21.26^{c}	3.296
18h00	14.07^{b}	16.92^{ab}	23.31^{a}	24.33^{a}	23.30^{a}	2.901

[a,b] Row means with different superscript differ significantly (P <0.05)

[c,d] Row means with a different superscript tend to differ (P <0.10)

[1]±SE: Standard error

[2]NH_3-N (mg NH_3-N/100ml)

RAN concentration is highly variable depending on DMI, level of protein and RDP of the diet, assimilation of NH_3 by the rumen microbes, and absorption and passage of NH_3 from the rumen. There was a difference (P <0.05) in RAN concentrations between the 0% urea treatment and the 0.83%, 1.25% and 1.66% urea treatments. The average RAN values ranged from 13.09 to 21.04 mg NH_3-N/100ml, with the 0% urea treatment being the lowest and the 0.83% urea treatment the highest. These values were in general higher than the suggested minimum level of 5 mg NH_3-N/100ml required for optimal microbial growth (Stern & Hoover, 1979 & Kennedy & Doyle, 1992). Although this is the minimum value considered for microbial growth, Schwab *et al.* (2005) proposed, based on a summary of the literature, that RAN concentrations of 8-18 mg NH_3- N/100ml are needed to maximize the flow of microbial N from the rumen especially when diets are high in fermentable carbohydrates.

The 0% urea treatment had a lower (P <0.05) minimum RAN concentration than the 0.83% urea treatment, and the 0% urea treatment had a lower (P <0.05) maximum RAN concentration than the 0.83%, 1.25% and 1.66% urea treatments. Figure 4.8 indicates that RAN was lowest for all treatments at 06h00 and reached a peak at 12h00 (Treatment 1 and 2) and 18h00 (Treatment 3, 4, and 5). The 1.25% and 1.66% urea treatments had more drastic increases following the feeding times. This was expected as these two treatments had the highest urea levels. It was expected that RAN concentration would increase as urea increased. Mynardt *et al.* (2016) observed significant increases in concentrations of RAN as the level of urea supplementation increased. Kanjanapruthipong & Leng (1998) also observed increased RAN concentrations with an increase in urea supplementation. In that study RAN concentrations increased from 6.7 to 16.8 mg/100ml of rumen fluid when urea supplementation was increased from 10 g/sheep/day to 15 g /sheep/day. In this study,there was a quadratic relationship between urea inclusion levels and maximum RAN concentration as seen in Figure 4.9.

Optimal use of ammonia released from RDP (or NPN) will occur if protein and carbohydrate degradation in the rumen occurs simultaneously. Ammonia concentration in the rumen is expected to increase if a soluble protein or NPN source is consumed and there is not sufficient readily available energy for microbial protein production (Bartley & Deyoe, 1981). Feed crude protein is divided into three fractions based on the rate of degradation (NRC, 1985, 2001; Sniffen *et al*., 1992). Fraction A (NPN) is degraded rapidly, fraction B (true protein) is potentially degradable and fraction C is mostly unavailable protein (acid detergent insoluble crude protein). The 0% and 0.42% urea treatments contained more of the diet crude protein in the form of fraction B, which can explain why there is a lower RAN concentration, and potentially a more synchronous degradation of protein and energy.

Although the starch levels increased as the urea levels increased in the treatment diets, it does not seem to have an effect on the rumen ammonia nitrogen concentration. Several reviews support the concept that the value of a synchronous supply of energy and protein, in theory, is not supported by experimental results (Cole& Todd, 2008; Hall & Huntington, 2008; Reynolds & Kristensen, 2008).

Another possible explanation might be the slower release of energy from starch granules compared with the rapid solubilization of urea and the release of ammonia. Sinclair *et al.* (1993) did *in sacco* trials which suggested the rate of starch fermentation takes longer than sugar fermentation. Thus the amount of degraded protein may have exceeded the amount of available fermentable energy, and the excess degraded protein was converted into ammonia nitrogen (McDonald *et al.,* 2011). Sinclair *et al.* (1995) observed peak rumen ammonia nitrogen concentrations one hour after feeding, however, rumen ammonia nitrogen was 60% higher in sheep receiving an asynchronous diet compared with a synchronous diet. However, RAN dropped three hours after feeding, while rumen pH was at its lowest at this point. Sinclair *et al*., (1993)suggested that starch disappearance from ground maize can take up to five hours to complete compared with the instantaneous disappearance of urea.

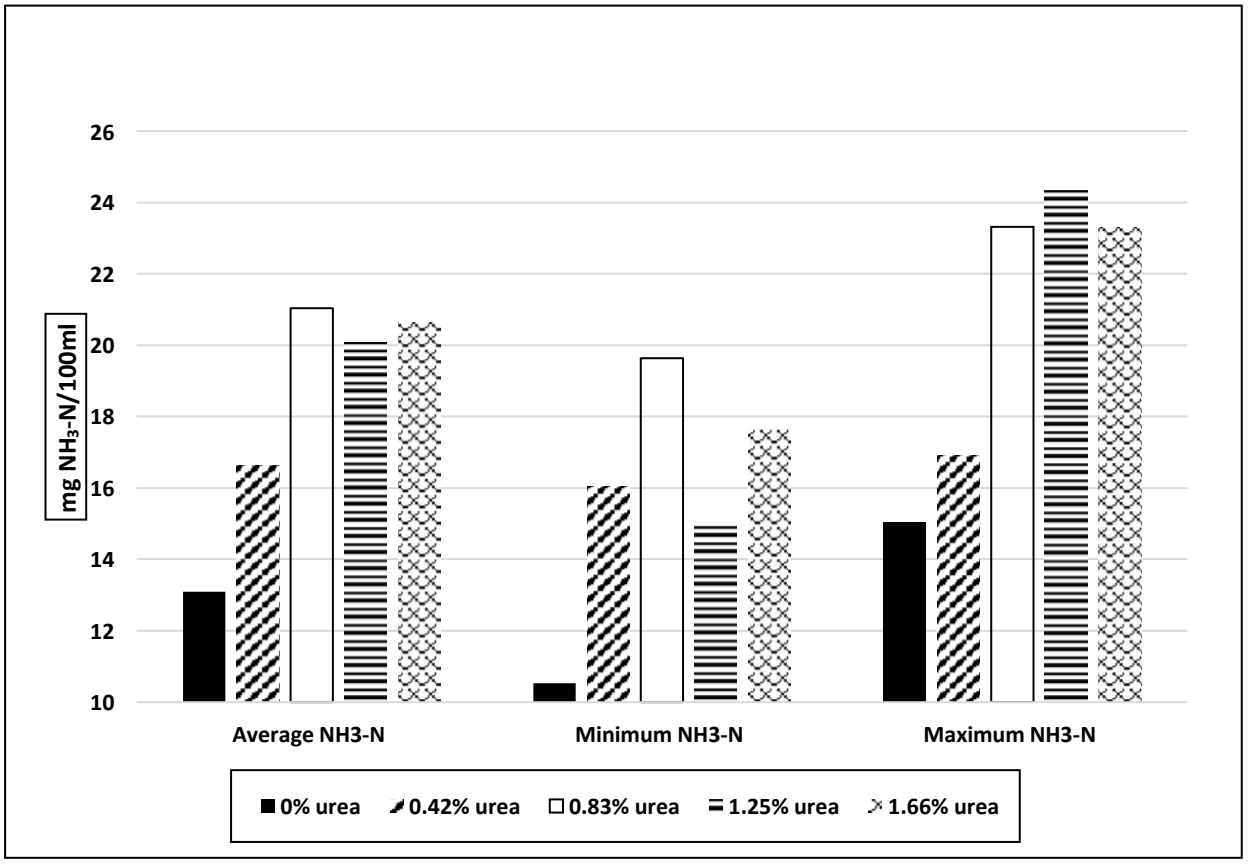

Figure 4.7 The effect of incremental levels of urea on rumen ammonia nitrogen concentration.

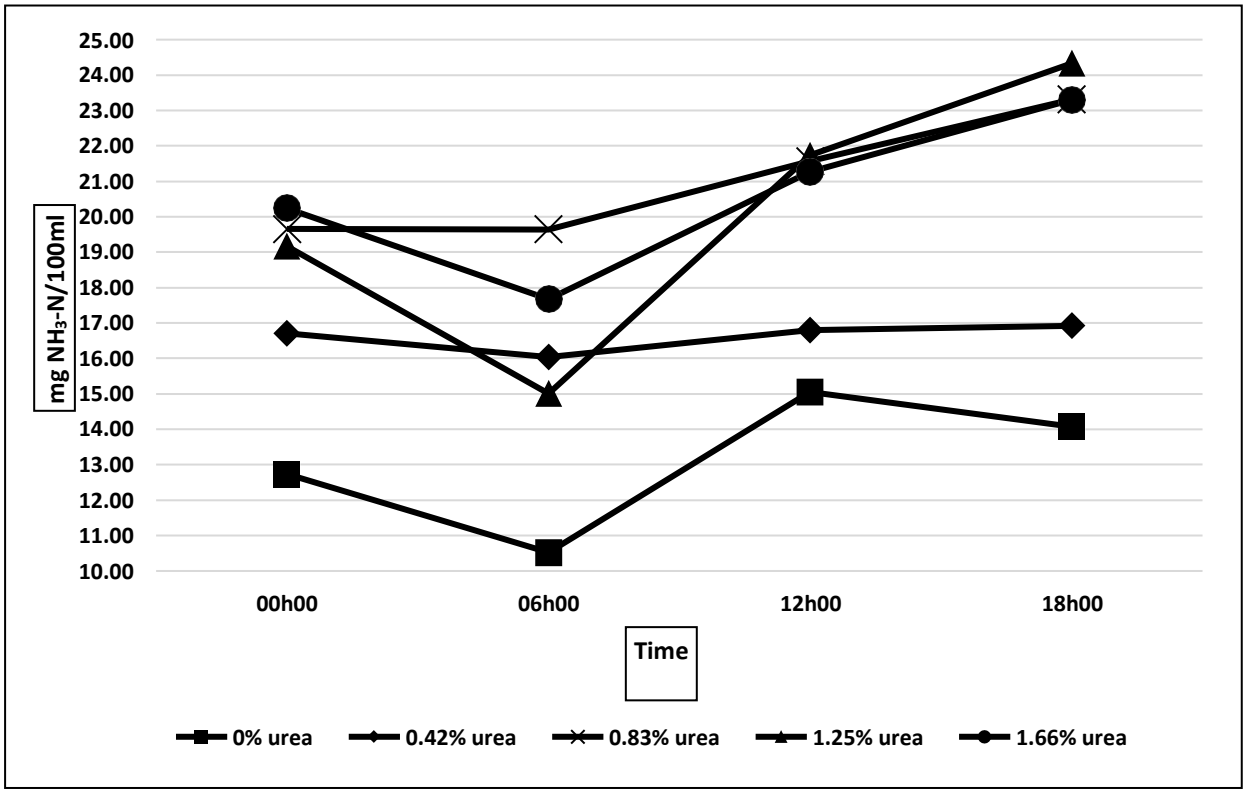

Figure 4.8 The rumen ammonia nitrogen concentration of sheep (over 24 hours) supplemented with different levels of urea.

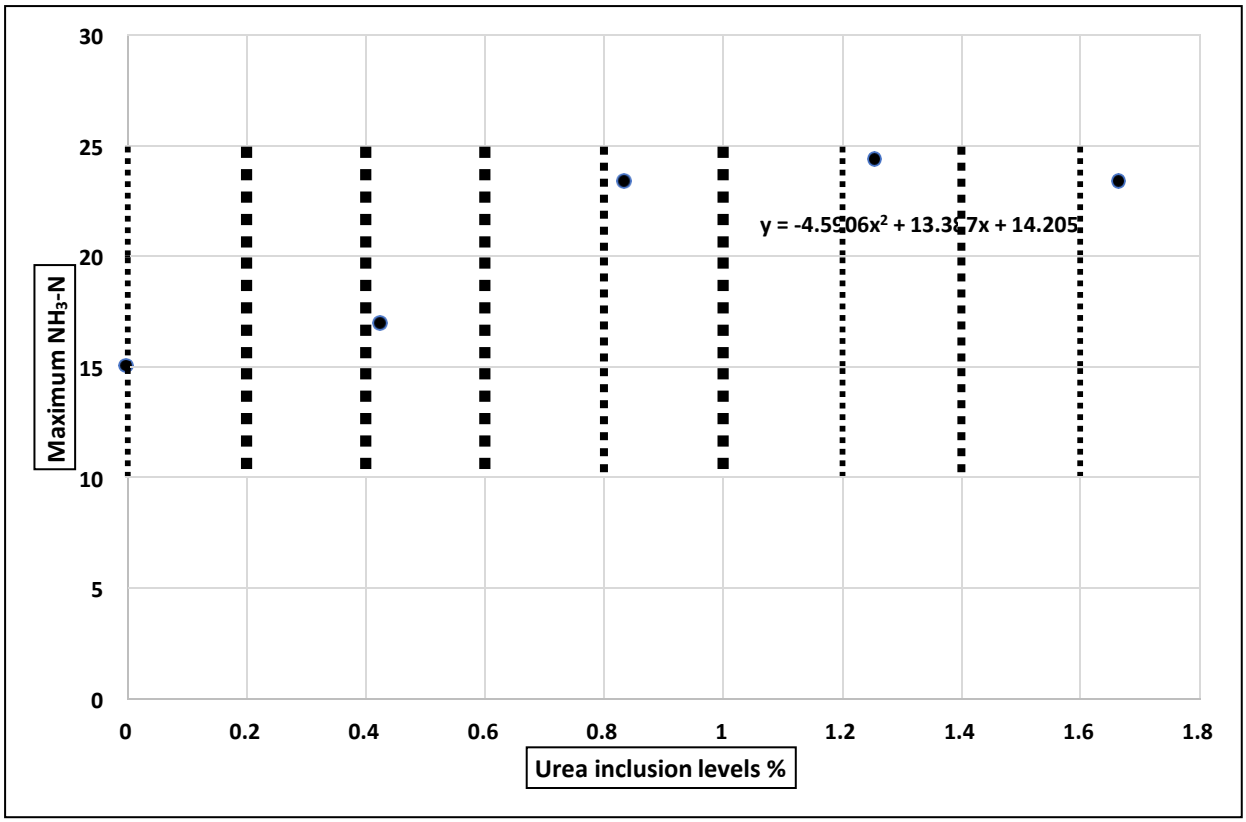

Figure 4.9 The quadratic relationship (P <0.05) between urea inclusion levels and maximum rumen NH_3-N.

4. Conclusions

The results from this experiment suggest that level of urea inclusion does affect the overall digestibility. There was an increase (P <0.05) in the digestibility coefficients of DM, OM, CP, and energy as urea levels increased. These findings were in agreementwith other studies in which ruminants received incremental levels of urea. The NDF digestibility of the 1.25% urea treatment was the lowest, which was due to the significantly lower (P <0.05) pH of that treatment. Apparent starch digestibility did not differ significantly between treatments (P >0.05). The high starch digestibility reported in this experiment were in general similar to other studies in which animals received processed and pelleted diets.

The incremental levels of urea did seem to have an effect on rumen fluid pH. As urea levels increased there was a drop in pH for treatments 1 to 4. This was the result of higher levels of starch. The VFA concentrations were not analyzed in this trial. This would have explained the rumen pH measurements observed. The 1.25% urea treatment had a significantly lower (P <0.05) average and minimum pH, and more time spent (P <0.05) under pH 5.5. The treatment with 1.66% urea, however, did not follow the same trend and did not differ from the 0% urea treatment in terms of average pH, minimum pH, and time spent under pH 5.5, but was lower (P <0.05) than the 0% urea treatment in terms of maximum pH.

RAN concentration was lower (P <0.05) for the 0% urea treatment compared with the 0.83%, 1.25% and 1.66% urea treatments. The 0% urea treatment had a lower (P
<0.05) minimum pH than the 0.83% urea treatment, and a lower (P <0.05) maximum pH than the 0.83%, 1.25% and 1.66% urea treatments. This did not have an effect on the digestibility study, as it was more than the minimum level of 5mg NH_3-N/100ml required for microbial growth.

The concluded result from this trial is that urea supplementation does have an effect on the overall rumen fermentation dynamics. The effects observed were not only the result of higher urea levels but also the effect of higher starch levels on rumen fermentation.

Chapter 5

General Discussion and Conclusions

This study was conducted to evaluate the typical feedlot finishing diets fed to lambs in South African feedlots. The objective of this study was to investigate the effects of incremental levels of urea on production parameters. The lambs sourced for the production study were homogenous and a close representation of the type of animals that are being fed in South African feedlots. Incremental levels of urea did appear to have an effect on growth performance.

The rumen fermentation study was conducted to evaluate the influence of incremental levels of urea supplementation on rumen fermentation dynamics and support or better explain the results obtained in the production study. Incremental levels of urea did have an effect on the digestibility coefficients. There was an increase in DM, OM, CP, and energy digestibility as the urea levels increased. This wasnot only the result of higher urea levels. Although diets were formulated on an iso-nitrogenous, iso-energetic, and iso-fibrous basis, the starch levels did differ between treatment diets. As urea was increased, the inclusion of maize was also increased and this resulted in decreased NDF digestibility. This is in agreement with several other trials in which ruminants received similar diets. The cannulated animals that were used were between 4 and 5 years old, thus they had fully developed rumens compared with the lambs. Therefore it's difficult to explain the feedlot performance from the results obtained in the 5x5 Latin square study. The rumen walls of the cannulated sheep weredamaged due to previous trials. This had an effect on the removal of VFAs from the rumen and resulted in overall low rumen pH measurements throughout the trial. The RAN concentrations for this trial were in agreement with similar studies. Due to cost implications, it wasn't possible to analyze all RAN samples, which possibly masked thetrue variation occurring shortly after feeding periods.

In conclusion, results from this study suggest that level of urea supplementation did have an effect on lamb feedlot performance. The rumen fermentation study, a 5x5 Latin square design, provided results that failed to explain the better performance obtained, based on ADG and FCR, of lambs receiving the 0% urea diet.

Implications

In this trial, urea was included at the expense of COCM, but although the diets were formulated on an iso-nitrogenous, iso-energy, and iso-fibrous basis, the starch levels did vary a lot between the different treatment diets. Therefore the results obtained in both the feedlot trial and the Latin square design could not only have been ascribed to the incremental levels of urea. This means that this trial was not only a study on the effect of protein quality but also the effect that different starch levels have on feedlot performance and rumen fermentation dynamics.

The animals used in the feedlot trial were young lambs with rumens that were not yet fully developed. This means that the results obtained from the wethers used in the rumen fermentation study were not truly representative to describe the results obtained in the feedlot study. Urea is an NPN that can be converted by rumen micro-organism into protein available for the animal. This is only possible if the rumen is fully developed. Urea that is not converted by microbes into protein will have to be excreted by the animal. This process whereby urea is either excreted or recycled is anenergy tapping process. Therefore young lambs might not have efficiently utilized the high levels of urea and had to excrete the excess. Therefore these animals had less energy available for growth. Unfortunately, there were not enough funds available toanalyze the urine in the Latin square design. This might have given a better indicationof the efficiency of urea utilization as opposed to CP digestibility alone.

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