# Effect of dietary protein quality and rumen protected amino acid supplementation on performance and carcass characteristics of feedlot cattle.

by

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Submitted in fulfillment of the requirements of the degree.

**MSc (Agric) Animal science: Animal Nutrition**

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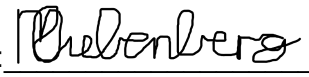
# Declaration

I, Marnus Liebenberg, do hereby declare that the research presented in this thesis, was conceived, and executed by myself, and apart from the necessary guidance from my supervisors, I have received no assistance. Neither the substance, nor any part of this thesis has been submitted in the past at this or any other University. This thesis is presented in partial fulfilment of the requirements for the degree MSc (Agric) Animal science: Animal Nutrition. I hereby grant the University of Pretoria free license to reproduce this dissertation in part or as whole, for the purpose of research or continuing education.

Signed: 

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# Abstract

The objective of this study was to evaluate the effect of rumen undegradable protein (RUP) and rumen protected lysine (RPLys) and methionine (RPMet) supplementation on growth performance, blood parameters and carcass characteristics of feedlot cattle over a 135-day feeding period. One hundred and twenty Bonsmara type steers were blocked by body weight and randomly allocated to one of four treatment diets in a complete randomised block design. Each treatment consisted of 5 pens with 6 animals per pen. The treatments were 1) Basal diet supplemented with urea (CON), 2) Basal diet supplemented with RUP (RUP), 3) Basal diet supplemented with RUP and RPLys (RUP+L) and 4) Basal diet supplemented with RUP and RPLys and RPMet (RUP+L+M). Data was statistically analysed using the PROC MIXED model (SAS, 2021). The average daily gain (ADG) and feed conversion ratio (FCR) did not differ between treatments and ranged from 1.84 to 1.90 kg/d and 4.84 to 5.04 kg feed / kg gain respectively ($P>0.05$). Treatments RUP+L and RUP+L+M resulted in lower serum blood urea nitrogen (BUN) concentrations on three sampling days when compared to the CON treatment which is indicative of better N utilisation ($P<0.05$).Serum blood AA were reduced for Ala, Glu, Pro and Tyr on sampling day 99 and reduced for Ileu, Lys, Leu, Val , Pro and Tyr when treatment RUP+L+M is compared to CON suggesting an increased uptake and utilisation of these AA for protein and muscle deposition ($P<0.05$). The 13$^{th}$ rib subcutaneous fatness was lower for treatment RUP+L+M compared to the other treatments ($P < 0.05$) and accordingly the channel fat mass of carcasses from animals supplemented with both RPLys and RPMet was lower than the CON group ($P<0.05$). This suggest that Lys and Met supplementation as well as the ratio of Lys to Met may be important factors to consider if the consumer demands leaner carcasses. In conclusion, a diet formulated according to NRC standards with sufficient RDP, energy and effective NDF that promotes optimal rumen fermentation can achieve above average growth performance and do not need to be supplemented with additional RUP or RPAA to meet MP requirements. Supplementation of RPLys and Met has shown potential for production of leaner carcasses.

# Chapter 1
# Introduction

It is generally accepted by feedlot nutritionists that protein quality is important in feedlot nutrition but in practice little attention is given to protein quality or amino acid (AA) requirements when formulating diets. Formulation on a crude protein (CP) basis with some emphasis on rumen undegradable protein (RUP) and AA supply is common (Vasconcelos & Galyean, 2007; Erickson *et al*., 2016). There is, however, sufficient evidence that maize-based diets with non-protein nitrogen (NPN) as the primary protein source do not fulfil the metabolizable protein (MP) requirements during the early stages of growth, namely the initial receiving and early growing period (Zinn, 2014; Torrentera *et al*., 2016).

During the late grower and finisher phases dry matter intakes (DMI) are higher than the starter phases, and microbial AA plays a more dominant role in fulfilling the maintenance and growth requirements of cattle. These requirements are met to the extent that the rumen degradable protein (RDP) content of the diet may be the only significant formulation factor considered with regard to protein nutrition (Zinn, 2014). Limited research is available on the effect of protein quality during the different feeding phases in the feedlot, and the question of up to which point during the feeding period protein quality impacts growth performance, remains.

In a review of AA nutrition of feedlot calves, Zinn (2014) stated that the only area where higher protein and RUP supplementation of feedlot cattle has consistently enhanced growth performance has been during the receiving and the early grower feeding phases. This period may vary from as little as 7 days in yearling cattle to 28 or even 56 days in lighter weight calves. In the special case of calf-fed Holsteins this phase may extend until approximately 112 days (Ainslie *et al*., 1993; Ludden *et al*., 1995, Klemesrud *et al.,* 2000; Zinn *et al*., 2007).

It has been reported that increased levels of RUP significantly improved the performance of growing cattle fed either a total mixed ration (TMR), grazing forages as well as in backgrounding cattle (Erickson *et al*., 2016; Tibbitts *et al*., 2016; Hilscher *et al.,* 2016). In these studies, the sources of RUP and RUP amino acids were soybean meal, heat treated soybeans, dried distillers' grain and maize gluten 60.

Feedlot cattle are different than backgrounding cattle as diets are based mainly on maize and maize by-products. Much of the research on finishing cattle targeted RDP supplementation to maximize gain and feed efficiency (Shain *et al*., 1998; Cooper *et al*., 2002). Very few studies have evaluated RUP supplementation as maize is relatively high in RUP and it is expected to supply sufficient RUP, except early in the feeding period (Erickson

*et al*., 2016). Zinn *et al.* (2000) fed cattle two isonitrogenous diets containing 20% NPN or 40% NPN of dietary N by partial replacement of urea N with fishmeal N. Decreasing the NPN: N ratio increased ADG by 36% and 40% respectively for the initial two 56-day periods of the trial. Hussein and Berger (1995) found that a diet containing soybean meal only compared to a diet containing a 50:50 mixture of urea and soya improved performance during the first 84 days of the trial. In other studies where urea was replaced by a blend of animal products of soybeans or rumen protected soybeans, the natural protein source diets improved average daily gain and feed conversion for the first 56 days and first 28 days on feed respectively (Zinn & Owens, 1993, Ludden *et al.,* 1995).

Deficiencies in essential AA in the early growing phase have shown a negative influence on ADG and DMI (Hussein & Berger, 1995; Wessels *et al.,* 1997). Research on the AA requirements of growing cattle states that methionine (Met) is the primary limiting AA with lysine (Lys) the second. With few exceptions (i.e. fishmeal) protein supplements commonly fed to feedlot cattle (oilseed meals, maize by-products) are not good sources of methionine and /or are deficient in lysine (Wessels *et al.,* 1997). There is therefore renewed interest in the role of rumen protected AA (RPAA) in the nutrition of feedlot cattle during the early growth phases (Prestegaard *et al.,* 2017; Torrentera *et al.,* 2016). Compared to dairy cattle limited research has been published on the supplementation of RPAA to feedlot cattle. In some studies, RPAA (Lys and Met) improved performance (Torrentera *et al.,* 2016; Prestegaard *et al.,* 2017) but in others no responses were found (Oney *et al.,* 2016, Texeira *et al.,* 2019).

Limited South African research is available on the effect of feed protein quality on feedlot cattle performance during the early growth phases (starter phase). Meissner *et al.* (1992) investigated the optimum levels of CP and RUP in feedlot diets and recommended 35-40% of total protein supply should be from RUP with CP levels declining from 14% in the early growth phase to 10% (DM) in the finisher phases (day 64-130). These authors however did not investigate the role of different sources of RUP or differences in AA profiles. The South African feedlot industry therefore relies mainly on research from the USA, which is not always comparable to South African feedlots since feedlot cattle entering the USA feedlots at a higher initial body weight (BW) (272-363kg) and age and the feeding period is also longer with a mean days on feed of 201 (Samuelson *et al.*,2016).

The aim of the study was to investigate whether protein quality of RUP and rumen protected AA (RPAA) supplementation should be considered when formulating feedlot cattle diets under SA conditions. The objectives of this study are:

1. To determine the effect of protein quality (RUP, AA profile, RPAA (Rumen protected amino acids) on the growth performance of feedlot cattle during the starter, grower, finisher and overall feeding period, respectively.
2. To determine the effect of protein quality on blood parameters, including blood urea nitrogen (BUN), free fatty acids (FFA), amino acid profile and creatinine.
3. To determine the effect of protein quality on carcass characteristics.

We hypothesized that protein quality and rumen protected amino acid supplementation will improve the growth performance of feedlot steers, especially during the starter and grower phases.

# Chapter 2
# Literature Review

## 2.1 Introduction

The world population is projected to reach 9.74 billion by 2050, emphasising the important role that animal scientists will play in feeding this population (FOASTAT, 2020). According to the FOA (Food and Agriculture Organization of the United Nations) the world food production will have to increase by 70% in order to feed the human population by 2050. Meat production will have to increase by 200 million tonnes to feed the growing population, while land availability is becoming an problem. Extensive red meat production will play a less prominent role in future since more intensified meat production systems will be needed.

The most effective way to optimally produce red meat (beef) is through intensive feedlot systems. In South Africa 75-80% of its red meat is produced in feedlots (Olivier, 2021). The capacity of SA feedlots is 750 000 head with a throughput of 2 630 000 head per annum. The average mass of a calf entering the feedlot is 253kg and the average slaughter mass is 465kg resulting in an average carcass mass of 272kg. Statistics on feedlot efficiency is as follows: Average daily gain (ADG) = 1.7kg/d, Feed conversion ratio (FCR) = 5.5 kg feed/kg gain, mortality = 0.8%, dressing percentage is 58.5% and 135 days on feed (Olivier, 2021). Improving the efficiency of red meat production with limited resources will go a long way to help improve food security and profit for the producer.

In South Africa, the consumer prefers the meat to be on the leaner side, hence farmers drive to produce leaner tissue. Nighty five percent of meat produced in commercial feedlots is A grade and 5% is AB grades (Olivier, 2021). To produce leaner carcasses while still reaching a carcass weight of 250kg-300kg, the farmer and nutritionist must pay careful attention to the feed formulation. One of the important aspects is feed protein quality and the AA requirements of the animal. These effects, needs to be researched further, especially in younger animals during the early growth phases (Zinn, 2014). Feedlot cattle is fed via three phases, a starter, grower, and finisher. The roughage content is decreased from the starter to the finisher, while the grain inclusion is increased from the starter to the finisher, shifting the bacteria population from fibrolytic to amylolytic bacteria. The CP content generally decreases from 14% to 10% (DM) and the ME content increases from 11.3 to 12.5 ME/kg DM during the different growth phases (Feedlot manual, 2002)

Protein quality has only in recent years become a more prevalent concept in South African feedlot diets. Although the focus on protein quality is important in feedlot nutrition, there is limited research available on the practical application of protein quality concepts in diet formulation. Most of the data available is from studies conducted in the USA, which cannot

be directly extrapolated to South Africa as our climate and other production criteria for example days on feed, intake weight and slaughter weight are different (Sameulson *et al.*, 2016; Cowley *et al.*, 2019).

The importance and impact of protein quality and AA supplementation in feedlot diets under South African conditions is unknown and require investigation. In addition, the breakpoint in the early feeding phases where protein quality has a lesser impact on performance as well as the issue of whether there is a carryover effect to the later feeding phases are additional research questions to be addressed.

In the following sections protein utilization in ruminants, the role of RDP, RUP and AA as well as factors affecting microbial protein production will be discussed. In addition, the impact of protein quality during the different feedlot phases will be discussed. The impact of protein quality on carcass characteristics will be investigated in this study and therefore this section is concluded with a discussion on the beef carcass classification system.

## 2.2 Why focus on protein quality?

Ruminant animals have the unique capability to breakdown and degrade plant material that is indigestible to monogastric animals through microbial fermentation in the rumen. This can be a double-edged sword as no matter what quality protein source is fed, ruminants will convert all soluble protein and a variable part of the insoluble protein to the to the same breakdown products, ammonia, AA, peptides and carbon. Different protein fractions can be distinguished in numerous feeds since not all protein is 100% rumen degradable. Feeds are therefore categorised according to degradability and different feeds contain variable fractions of rumen degradable protein (RDP) and rumen undegradable protein (RUP) (NASEM,2016)

Protein quality is a term used in ruminant nutrition to explain the rumen degradability of the protein source and the amino acids provided by the protein source. Generally, RUP is a better-quality protein as it may provide limiting amino acids not synthesized in sufficient quantities by the rumen. It is also considered that a higher quality protein has a higher RUP fraction (Chalupa, 1975)

In the past limited emphasis was placed on protein quality but rather on the crude protein (CP) content of the diet. For this reason, feedlot nutritionist preferred feedstuffs with a high RDP content as they are more cost effective and still provides the animal with protein (ammonia). The cheapest source of them all is non protein nitrogen (NPN) sources like urea. Feedlot nutritionists would normally formulate diets with the available energy sources such as maize and maize by-products, then bring the CP up to the desired levels with the urea supplementation (Sameulson *et al.*, 2016).

Only feeding to CP specifications may lead to overfeeding of Nitrogen (N), this may have immense consequences on the environment. All the N that is not utilized by the animal gets excreted in the urine and faeces. The N gets volatilised to ammonia (NH3) and nitrous oxide ($N_2O$) where these 2 compounds follow a whole cycle as described by Cowley *et al*. (2019) until it leads to soil acidification, eutrophication of the ecosystem which leads to a loss in biodiversity (Cowley *et al.*, 2019). According to Beusen *et al*. (2008), agriculture is responsible for 32 T/year of NH3-N emissions with livestock especially in intensive systems, contributing 59-71% of these NH3-N emissions. With this in mind, it is imperative that nutritionists, through precision feeding, compare the efficiency of N utilization when meeting the animals requirements. This will contribute to maximising the growth potential of the animal and reducing excess N excretion into the environment.

## 2.3 Protein quality during the feedlot phases

In South Africa, similar to large parts of the USA, cattle feedlot diets are based on maize and maize by products with urea as the primary source of nitrogen (N). These diets generally contain 14% crude protein (CP) during the starter phase and 12-13% CP during the grower and finisher phases (Vasconcelos *et al.,* 2007; Zinn *et al.,* 2007; NASEM, 2016). In theory these diets satisfy the metabolizable amino acid (MAA) requirements during the total feedlot feeding period but may not fulfil the nutrient requirements during the early stages of growth (Zinn *et al*., 1998; NRC, 2001). During the starter and early growing period, feed intakes are low relative to the genetic potential of the calf for growth and therefore more emphasis should be placed on rumen undegradable protein (RUP) and amino acids to fulfil requirements since microbial AA play a lessor role during these growth phase (McDonald *et al*., 2011)

During the late grower and finisher phases dry matter intakes (DMI) are higher and microbial AA plays a more dominant role in fulfilling the maintenance and growth requirements of cattle. In these phases the rumen degradable protein (RDP) content of the diet may be the only significant formulation factor considered with regard to protein nutrition in order to supply the rumen microbes with ammonia (Zinn, 2014). Up to which point protein quality impacts growth performance in feedlots have limited research available and is still an important point of debate amongst nutritionist and feedlot managers. Insufficient research is available on the effect of protein quality on feedlot cattle performance during the early growth phases (starter phase) in South African. Meissner *et al.* (1992) investigated the optimum levels of CP and RUP in feedlot diets and recommended 35-40% RUP with CP levels declining from 14% in the early growth phase to 10% DM in the finisher phases (day 64-130). These authors however, did not investigate the role of different sources of RUP or differences in AA profiles. The South African feedlot industry therefore relies mainly on research from the US, which is not always comparable to South African feedlots since feedlot cattle enter US feedlots at a

higher initial body weight (BW) and age and the feeding period is also longer (Sameulson *et al.*,2016).

In a review of AA nutrition of feedlot calves, Zinn (2014) stated that the only area where higher protein and RUP supplementation of feedlot cattle has consistently enhanced growth performance has been during the receiving and the early grower feeding phases. This period may vary from as little as seven days in yearling cattle to 28 or even 56 days in lighter weight calves. In the special case of post weaned Holstein calves this phase may extend until approximately 112 days (Ainslie *et al*., 1993; Ludden *et al*., 1995, Klemesrud *et al.,* 2000; Zinn *et al*., 2007).

It has been reported that increased levels of RUP significantly improved the performance of growing cattle fed a total mixed ration, grazing forages as well as in backgrounding cattle (Erickson *et al*., 2016; Tibbitts *et al*., 2016; Hilscher *et al.,* 2016). In these studies, the sources of RUP and RUP amino acids were soybean meal, heat treated soybeans, dried distillers' grain and maize gluten 60.

Feedlot cattle are different than backgrounding cattle as diets are based mainly on maize and maize by-products, whereas backgrounding cattle relies mainly on natural veld/pasture for their feed with a lick supplement up to 1% of their body weight. Much of the research on finishing cattle targeted RDP supplementation to maximize gain and feed efficiency (Shain *et al*., 1998; Cooper *et al*., 2002). Very few studies have evaluated RUP supplementation as maize is relatively high in RUP and it is expected to supply sufficient RUP, except early in the feeding period (Erickson *et al*., 2016). Zinn *et al.* (2000) fed cattle two isonitrogenous diets containing 20% NPN or 40% NPN of dietary N by partial replacement of urea N with fishmeal N. Decreasing the NPN: N ratio increased ADG by 36% and 40% respectively for the initial two 56-day periods of the trial. Hussein and Berger (1995) found that a diet containing soybean meal only compared to a diet containing a 50:50 mixture of urea and soya improved performance during the first 84 days of the trial. In other studies where urea was replaced by a blend of animal products of soybeans or rumen protected soybeans, the natural protein source diets improved average daily gain and feed conversion for the first 56 days and first 28 days on feed respectively (Zinn & Owens, 1993, Ludden *et al.,* 1995).

Deficiencies in essential AA in the early growing phase have shown a negative influence on ADG and DMI (Hussein & Berger, 1995; Wessels *et al.,* 1997). Research on the AA requirements of growing cattle states that methionine is the primary limiting AA with lysine the second. With few exceptions (i.e. fishmeal) protein supplements commonly fed to feedlot cattle (oilseed meals, maize by-products) are not good sources of methionine and /or are deficient in lysine (Wessels *et al.,* 1997). There is therefore renewed interest in the role of rumen

protected AA in the nutrition of feedlot cattle during the early growth phases (Torrentera *et al.,* 2016; Prestegaard *et al.,* 2017). Compared to dairy cattle, limited research has been published on the supplementation of rumen protected amino acids (RPAA) to feedlot cattle. In some studies, RPAA (Lys and Met) improved performance (Prestegaard *et al.,* 2017; Torrentera *et al.,* 2016) but in others no responses were found (Oney *et al.,* 2016).

## 2.4 Protein utilization in ruminants

Ruminant animals have the unique capability to breakdown protein to nitrogenous substances like ammonia, peptides and AA which can then be used by microbes in the rumen to synthesize microbial protein. It is therefore important when formulating diets that a differentiation must be made between RDP and RUP. As described in Figure 1, the RUP fraction passes through the rumen into the abomasum and small intestine, where the digestible part will be absorbed as amino acids and used to synthesize animal tissue. The RDP fraction will either be rapidly degraded or slowly degraded, but both fractions will contribute towards ammonia, peptides and amino acids. These 3 constituents (ammonia, amino acids and peptides) will contribute towards microbial protein growth, giving the ruminant the ability to produce high quality protein from low quality protein sources. The microbial protein flows out of the rumen into abomasum and small intestine, where the microbial protein gets digested and absorbed via the small intestine. In addition to microbial protein is the balance of the MP flow to the small intestine supplied by the RUP fraction. This microbial protein fraction will become the most dominant protein source for ruminants from the grower phase and will play a considerable role in supply the animals amino acids need (McDonald, 2011).

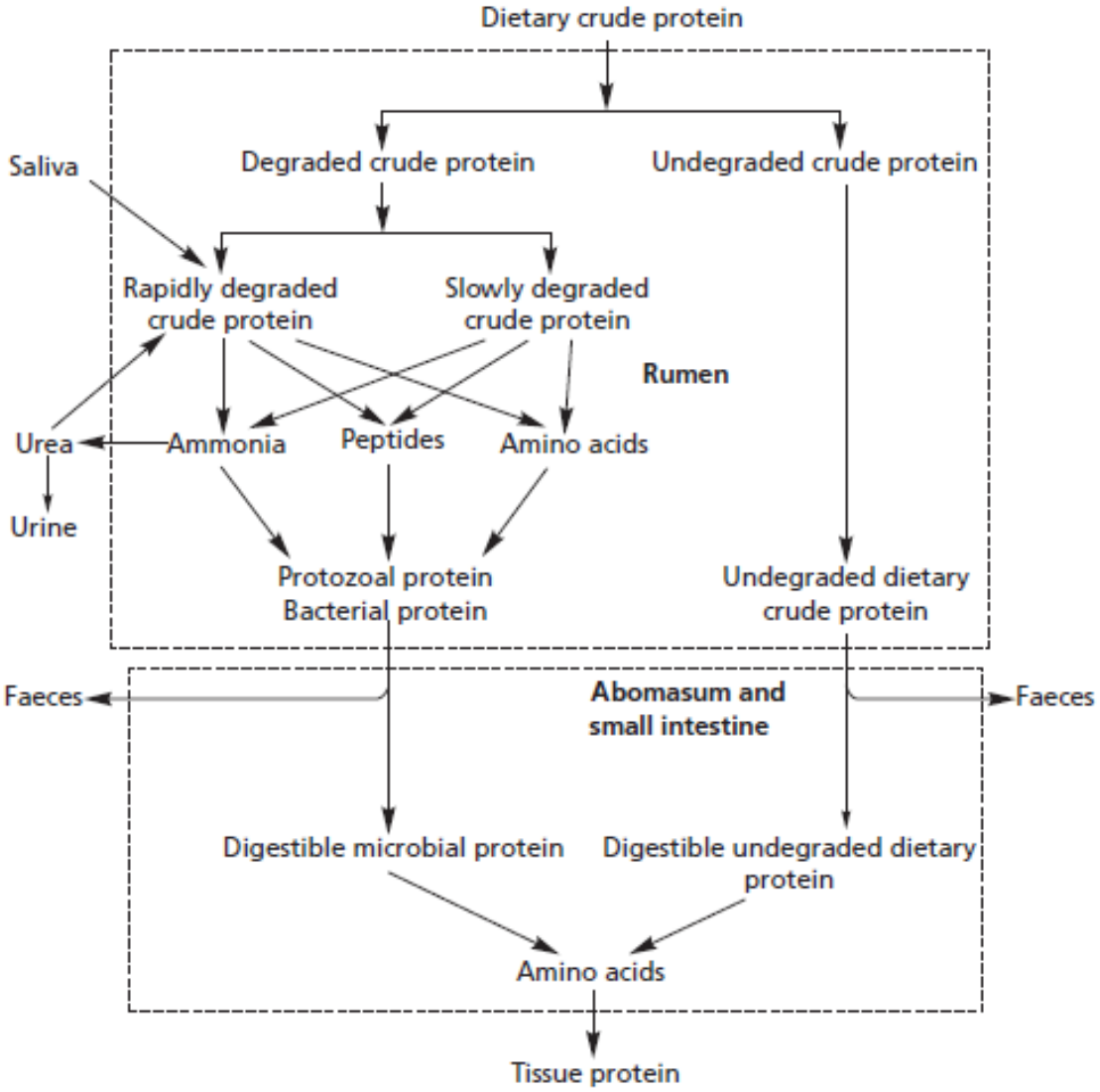



Figure 1:Crude protein degradation and utilization in ruminants (McDonald, 2011).

## 2.5 Rumen degradable protein (RDP)

The rumen degradable fraction is a difficult fraction to manage as RDP is mostly utilised by the rumen microbes for microbial protein synthesis. In order to ensure that the N from the RDP is efficiently utilized, RDP must be fed to meet the microbes demand for N but not to exceed the requirements, while supplying sufficient rumen fermentable carbohydrates to ensure optimum microbial production. An excess of RDP will result in excess urea reaching the liver which in then excreted via urine, leading to possible pollution. In extreme cases high levels can lead to toxicity as well (Schwab, 1995).

Microbes that ferment structural carbohydrates only need $NH_3$ as N source while bacteria that ferments non-structural carbohydrates need $NH_3$, peptides and AA as N sources.

## 2.6 Rumen undegradable protein (RUP)

When referring to different RUP sources it is also important to take differences in the AA profile into account. There are 20 amino acids that can be classified as essential amino acids (EAA) or non-essential amino acids (NEAA). The NEAA can be synthesized by the animal, usually from other AA or compounds whereas the EAA cannot be synthesized by the animal and must be provided through dietary intake to ensure normal function of the animal. Rumen undegradable protein is generally fed to complement microbial protein in the small intestine and to contribute in meeting the animals most limiting amino acid demands (Hvelplund, 1985; Rae and Smithard, 1985; de Boer *et al.,* 1987; Frydrych, 1992; McDonald, 2011).

In Table 1 is shown a comparison of the EAA profiles of lean tissue, rumen microbes and a variety of feedstuffs. The lean tissue fraction depicts the ideal relationship of the different essential ammino acids. The AA profile of rumen bacteria is very similar to that of lean tissue, especially lysine and methionine which are known to be first limiting for feedlot cattle and diary cattle under most feeding conditions (Wessels *et al.,* 1997). In diets with large proportions of maize for example, lysine is the first limiting AA for growing beef, because maize is low in Lysine (Xue *et al*, 2011). The aim should always be to optimize microbial production and the supplement with RUP feeds that complement the AA profile of microbes.

The RUP fraction of feeds such as blood meal, fish meal and soybean meal are good sources of lysine while fishmeal, maize gluten meal and brewers grains are good sources of methionine.

Clark *et al.,* (1992), summarized the data from 441 bacterial samples from animals fed 61 different dietary treatments in 35 studies and noted that the AA profile is variable. The Lys component varied between 15,8 - 17,3% and the Met varied between 4,9 - 5,2% (Clark *et al.*, 1992), while the protozoa had a Lys content is 20,6% and Met content is 4,2% (Storm *et al*., 1983). The AA profile of the microbial protein is better matched to the lean tissue AA profile and therefore it would be better to rely on microbial protein to be the main protein source for the ruminant. Microbial protein can supply 50-75% of the total amino acid supply of ruminants (Dewhurst *et al*., 2000), emphasizing the importance of microbial protein. One option for supplying the optimal amino acid supply is to optimize microbial protein production and the supplement with rumen protected AA and accompanied by rumen protected amino acids, starting with the first 2 most limiting amino acids.

Table 1: Comparison of the EAA profiles of lean body tissue with that of ruminal bacteria, protozoa, and common feeds. (Adapted from Schwab,1995)

| | % Of total EAA | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | Arg | His | Ile | Leu | Lys | Met | Phe | Thr | Trp | Val |
| **Animal products** | | | | | | | | | | |
| Lean Tissue | 16.8 | 6.3 | 7.1 | 17.0 | 16.3 | 5.1 | 8.9 | 9.9 | 2.5 | 10.1 |
| **Rumen Microbes** | | | | | | | | | | |
| Bacteria | 10.2 | 4.0 | 11.5 | 16.3 | 15.8 | 5.2 | 10.2 | 11.7 | 2.7 | 12.5 |
| Protozoa | 9.3 | 3.6 | 12.7 | 15.8 | 20.6 | 4.2 | 10.7 | 10.5 | 2.8 | 9.7 |
| **Forages** | | | | | | | | | | |
| Lucern | 10.9 | 5.2 | 10.9 | 18.4 | 11.1 | 3.8 | 12.2 | 10.6 | 3.4 | 13.5 |
| Maize silage | 6.4 | 5.5 | 10.3 | 27.8 | 7.5 | 4.8 | 12.0 | 10.1 | 1.4 | 14.1 |
| Haycrop silage | 8.9 | 5.3 | 11.0 | 18.9 | 10.3 | 3.8 | 13.5 | 10.3 | 3.3 | 14.7 |
| **Grains** | | | | | | | | | | |
| Barley | 12.8 | 5.9 | 9.6 | 18.4 | 9.6 | 4.5 | 13.3 | 9.1 | 3.1 | 13.6 |
| Yellow maize | 10.8 | 7.0 | 8.2 | 29.1 | 7.0 | 5.0 | 11.3 | 8.4 | 1.7 | 11.5 |
| Oats | 15.6 | 5.4 | 9.5 | 18.1 | 10.0 | 4.3 | 11.5 | 9.2 | 3.2 | 13.3 |
| Sorghum | 9.4 | 5.8 | 9.4 | 30.9 | 5.6 | 4.3 | 12.6 | 8.0 | 2.2 | 11.8 |
| Wheat | 15.2 | 6.6 | 9.7 | 18.9 | 8.0 | 4.6 | 12.6 | 8.3 | 3.4 | 12.6 |
| **Plant Proteins** | | | | | | | | | | |
| Brewer’s grain | 8.9 | 6.4 | 10.6 | 17.6 | 11.4 | 4.8 | 10.3 | 11.4 | 3.0 | 15.6 |
| Maize gluten meal | 6.9 | 4.7 | 9.3 | 36.4 | 3.8 | 5.5 | 13.8 | 7.5 | 1.5 | 10.7 |
| Cottonseed meal | 25.4 | 6.0 | 7.7 | 13.9 | 9.6 | 3.8 | 12.2 | 7.7 | 2.9 | 10.8 |
| Soybean meal | 16.3 | 5.7 | 10.8 | 17.0 | 13.7 | 3.1 | 11.0 | 8.6 | 3.0 | 10.6 |
| Sunflower meal | 19.4 | 5.9 | 10.1 | 15.5 | 8.6 | 5.4 | 11.0 | 9.1 | 2.8 | 12.3 |
| **Animal proteins** | | | | | | | | | | |
| Blood meal | 7.6 | 11.2 | 2.1 | 22.8 | 15.7 | 2.1 | 12.3 | 8.1 | 2.7 | 15.4 |
| Feather meal | 14.7 | 1.1 | 10.0 | 29.3 | 3.9 | 2.1 | 10.0 | 10.5 | 1.5 | 17.1 |
| Fish meal | 13.1 | 5.7 | 9.3 | 16.5 | 17.0 | 6.3 | 8.8 | 9.5 | 2.4 | 11.3 |

## 2.7 Factors affecting microbial protein

## 2.8 Factor affecting microbial protein production:

Microbial protein can account for up to 75% of the total amino acid supply for ruminants, and it is therefore of most importance to maximize microbial production (Sniffen & Robinson, 1987; Dewhurst *et al*., 2000; Broderick & Reynal, 2009). It is not only about microbial production but also the efficiency of microbial production. Bacteria with a faster growth rate has less maintenance energy compared to bacteria with a slower growth rate.

The following factors can affect microbial protein production:

### 2.8.1 Level of feeding

Feeding animals less frequently will result in a longer retention time in the rumen, resulting in a decrease in the efficiency of microbial protein synthesis as the microbes will have a higher maintenance cost. This is also accompanied by feeding higher levels of fibre in the diet as fibre will spend a longer time in the rumen (Robinson *et al*., 1985). Total feed intake plays a crucial role in ruminal pH, determining the survival of rumen microbes and which type of population occurs (Krause & Oetzel, 2006)

### 2.8.2 Rumen environment

Creating a favourable rumen environment for the most efficient microbial protein synthesis can be challenging since many factors (Feeding times, climate, feed mixing, type of ingredients etc.) can contribute to disturb the fermentation pattern in the rumen. To balance the rumen environment a highly fermentable energy source must be fed with a highly degradable protein supply in order to provide a synchronized energy and nitrogen supply to the rumen microbes. Effective fibre must also be a consideration in a ration to ensure optimal rumen function (Dewhurst *et al*., 2000).

Feeding high producing cattle is accompanied by increased starch levels in the diet. Increasing starch content can often have a negative impact on the rumen environment in terms of ruminal pH, decreased fibre degradation and possibly damage the rumen walls, decreasing absorption via the rumen wall (Russell and Wallace,1997). The solubility of the starch composition needs to be considered as small grains like wheat is more soluble (68%) whereas larger grains like maize is less soluble (26%). Large grains like maize generally have a larger rumen escape fraction of 40% versus small grains like wheat that has a rumen escape fraction of 7%. Hence it is important to remember that starch that is soluble in the rumen is energy for the microbes whereas starch that's flows through to the small intestine is energy for the animal

(Mills *et al*., 1999). This must be considered as rumen microbes need a readily fermentable energy source to grow efficiently (Overton *et al*., 1995).

Increasing energy levels in the diet with oil, above the maximum recommended level of 7%, can lead to a decrease in the efficiency om the rumen microbes. With high levels of oil the rumen microbes becomes encapsulated with oil inhibiting their ability to degrade feed (Ikwuegbu & Sutton, 1982; Tesfa,1993).

### 2.8.3 Forage quality

As noted earlier, higher forage diets will result is a longer retention time, decreasing microbial protein synthesis efficiency. The forage quality will also influence the efficiency as higher quality forage will result in an increase in microbial synthesis and lower quality forage will result in a decrease in microbial synthesis efficiency (Beever *et al*., 1986; Dove & Milne, 1994; Carruthers *et al*., 1997; Elizalde *et al*., 1998)

## 2.9 Feed grade urea in feedlot diets

Feed grade urea is known as a non-protein nitrogen (NPN) source and has been fed for ruminants for a very long time. Feed grade urea is only a nitrogen source and has no other nutritional component. When urea is converted to protein, the crude protein equivalent is 281%. The rumen microbes convert urea to ammonia and carbon dioxide. The ammonia either gets utilised by the microbes for microbial protein synthesis or gets transported to the liver where it is excreted through urine. Feeding to much urea can lead to a toxic build-up of ammonia in the liver (Hungate, 1966).

In order to optimally utilise urea, the microbes must have a sufficient supply of energy. Given the high energy content of feedlot diets, higher levels of urea can be utilised more efficiently. When feeding diets containing urea it is recommended to adapt the cattle to the urea diet and to split the urea supply by feeding at least two times a day. Aim not to exceed 33% of the ruminants total protein supply from urea. Broderick & Reynal (2009) found that replacing RDP from true protein with excessive levels of NPN reduced the animals performance.

When feeding high levels of urea degradability is much faster that the capability of the microbes to utilize the ammonia hence the use of slow-release urea has become more popular. Slow-release forms of urea is for example biuret, stearate, uromol, calcium chloride linked urea, urea-formaldehyde, urea-lignocellulosic complex, urea coated with a complex fat fatrix (Cherdthong *et al*., 2011; Steiner *et al*., 2019). Slow-release urea has become more popular especially the fat coated forms, as they can provide nitrogen for the rumen microbes as well giving the added benefit of energy.

## 2.10 Amino acid supply

### 2.10.1 The first limiting amino acids.

Lysine and methionine are the two most limiting amino acids of ruminants. Lean animal tissue consists of 16,3% Lysine and 5,1% Methionine as percentage of total essential amino acids (Ainslie *et al*., 1993). Yellow maize, that is universally used in high production rations has a Lys content of 7% and a methionine content of 5% expressed as a percentage of total essential amino acids. Soybean meal that is a frequently used protein source in ruminants has a Lys content of 13.7% and a Met content of 3,1% as a percentage of total essential amino acids (Schwab, 1995). It is clear that only taking the diet into account there can still be short comings in meeting the ruminant's amino acid requirements.

## 2.11 Why is undegradable protein more important?

When feeding a ruminant, the protein degradability will depend on a lot of factors. These factors include the type of preparation of the feed as well as animal factors like proteolytic activity in the rumen, retention time of the feed and ruminal pH (Kumar *et al*., 2015). Under normal feedlot condition the RUP fraction should be relative constant and will be able to reach the small intestine.

Feeding bypass protein, enable one to feed more specifically to a ruminant animal's needs. One will also be able to get essential amino acids to a ruminant without being broken down by rumen microbes and being used to build their own microbial protein.

Studies shown that only increasing the CP content of the diet doesn't necessarily resolve protein deficiencies (Broderick *et al*., 1974). An increase in CP with the addition of RUP is important as this is where the animals' deficiencies lie, as proven by feeding bypass protein that may also increase performance of the animals (Broderick *et al*., 2009; Flis & Wattiaux, 2005).

One of the options to feed good quality bypass protein is by using bypass amino acids. These amino acids are designed to pass the rumen and dissolve at a lower pH of the small intestine. An example of bypass amino acids is Ajipro L (bypass lysine), this product has a 41% CP value and is 20.3% rumen degradable. It is also proven that supplementation of rumen protected amino acids must be done in moderation as an oversupply of one amino acid not only has financial implications, but it can also be antagonising to other amino acids (Harper et al.,1964; Heiderscheit & Hansen, 2020)

## 2.12 The Beef carcass classification system

Carcasses in South Africa are classified according to age, fat, conformation, damage and sex. The age classification is distinguished by roll marks with codes, A, AB, B and C (please see descriptions in Table 2).

Table 2: Age classification of beef carcasses in South Africa

| Grade | Meaning | Age |
|---|---|---|
| A | No Permanent incisors | 0-18 Months |
| AB | 1-2 Permanent incisors | 9-24 Months |
| B | 3-6 Permanent incisors | 25-31 Months |
| C | > 6 permanent incisors | > 32 Months |

Fat classification is an indicator of how much sub cutaneous fat is on the carcass. The thickness is measured on a cold carcass between the $10^{th}$ and $11^{th}$ rib, 50 millimetres (mm) from the median line. The classification ranges from 0 that has no fat to 6 that is excessively over fat, (please see Table 3 for description) (Feedlot manual, 2002).

Table 3: Fat classification of beef carcasses in South Africa

| Fat Code | Description |
|---|---|
| 0 | No visible fat |
| 1 | Very lean (0.0-1.0mm) |
| 2 | Lean (1.0-3.0mm) |
| 3 | Medium (3.1mm-5.0mm) |
| 4 | Fat (5.1mm-7.0mm) |
| 5 | Moderately over fat (7.1mm – 10.0mm) |
| 6 | Excessively over fat (>10.0mm |

## 2.13 Factors affecting carcass characteristics

### 2.13.1 Genetics

The type of carcass that is produced by the feedlots is due to an interaction between the genotype and the environment to give us a specific phenotype that we see (P=GxE). In Table 4 is shown a few of the carcass parameters and their heritability's. Heritability is an indication

of the effect of the environment on the genes associated with the phenotypic character. A higher heritability means that the environment has less of an influence on the expression of the gene, where a lower heritability means that the environment has a more prominent influence on the expression of the genes. (Hendrick *et al*., 1989). Hence the biggest improvement of a carcass trait via selection would be those with higher heritability

Table 4: Heritability ranges for carcass composition traits across species (Adapted from Irshad et al., 2013).

| Trait | Heritability[1] |
|---|---|
| Ultrasound muscle depth/area | Moderate - high |
| Ultrasound fat depth | Moderate – high |
| Carcass weight | Moderate – high |
| Carcass length | High |
| Dressing percentage | Low – moderate |
| Lean yield | Moderate – high |
| Lean : bone ratio | Moderate – high |

[1] (Low = 0-0.25; moderate = 0.25-0.5; high = 0.5-1)

### 2.13.2 Maturity type and age

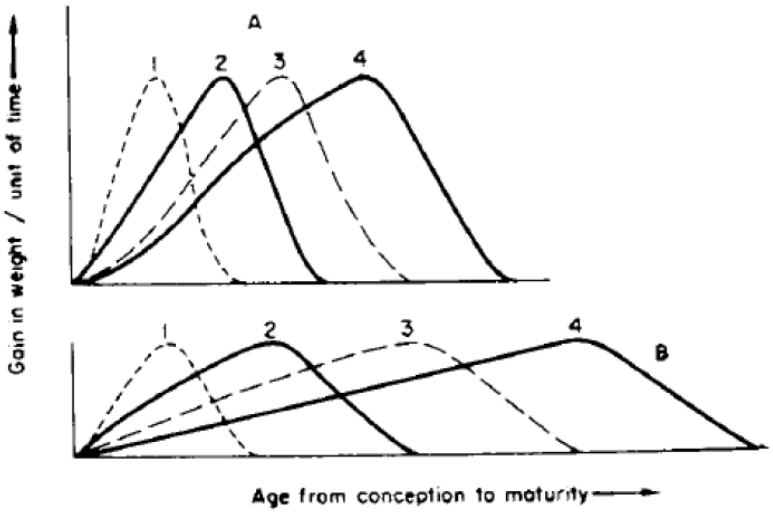
There is a difference in carcass composition between animal types (e.g. sheep and cattle) and between breeds (e.g. Bonsmara and Limousin). Breeds can be classed into different maturity types, that are generally divided in to early, mid, or late maturing types. Different maturity types would reach a certain physiological age at different chronological ages, as seen in Figure 2 where graph A depicts an early maturing breed and graph B depicts a later maturing breed.

Physiological age is used to describe a certain stage of development within an animal for example puberty or height. During the development of an animal, the shape and composition changes continually until maturity. As described by Hossner (2005) the difference in development stages can be seen in the head weight contributing to the animal's total weight, at birth the head contributes 20% of the total live weight whereas at maturity the head's weight contribute 5% to the total weight. As also seen in Figure 2 an animal will develop lean tissue earlier that fat tissue.

The maturity type determines how early an animal reaches physiological maturity. The closer an animal is to maturity the more energy is directed to fat growth (Figure 2). An early maturing breed reaches physiological maturity much earlier than a later maturing breed on a chronological scale. An early maturing breed can be slaughtered at a younger age compared

to a late maturing breed; early maturing breeds will also provide smaller carcasses than late maturing breeds. Early maturing cattle may be a good option when feed prices are high or feed is limited and late maturing cattle when feed prices are cheaper and more available (Kempster *et al*., 1986). Meat type breeds like a Bonsmara will have more sub cutaneous fat whereas milk producing breeds like a Jersey will have more internal fat (e.g. channel fat) at similar ages. In general, intramuscular fat is deposited after intermuscular fat. When comparing early maturing cattle with late maturing cattle, early maturing cattle has less intermuscular fat and more intramuscular fat (Irshad *et al*., 2013).

In South Africa a premium is paid for A2 and A3 carcasses, making it crucial for a farmer to produce a uniform product. For ease of management, farmers prefer an all-in all-out system in feedlots, meaning that the animals in a pen will be the same amount of days on feed ensuring an all-in all-out system. Grouping animals according to maturity type will be crucial in order to achieve a uniform carcass at the abattoir (Feedlot manual, 2002).




*A, early maturity or high plane of nutrition, B, late maturity or low plane of nutrition.*

| Curves | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| | Head | Neck | Thorax | Loin |
| | Brain | Bone | Muscle | Femur |
| | Cannon | Tibia-fibular | Femur | Pelvis |
| | Kidney fat | Intermuscular fat | Subcutaneous fat | Intramuscular fat |

Figure 2: The effect of maturity type and plane of nutrition on the growth rates of body regions and tissues during development. (Pállson & Hammond, 1955)

### 2.13.3 Sex

Female animals generally reach maturity sooner than intact males. Castrated males also tend to reach maturity sooner than intact males but still later than female animals, hence slaughtering the same breed at the same chronological time, female animals will have more fat than castrated males and castrated males will produce more fat than intact males. Male

animals also tend to have less tender meat due to the increased levels of intermuscular connective tissue (Irshad *et al*., 2013).

### 2.13.4 Nutrition

An animal can only express his full genetic potential when it receives a ration that has adequate amounts of protein, carbohydrate, fat, vitamins and minerals in the right ratios. The rate of growth will be dependent on the quality of the raw material fed to the animal (Hossner, 2005).

Although the animals' genes are determined when certain physiological developments take place as depicted in Figure 2, the animal's plane of nutrition will always dictate the rate of growth of the physiological developments as seen in Figure 2 where graph A depicts and animal on a high plane of nutrition and graph B an animal on a low plane of nutrition. The maintenance of the developed organs and tissue will always have priority over the development of new tissue. If an animal is placed on a restricted diet after maturity, utilisation would occur in the opposite direction as when it was formed, for example fat will be utilised first, then muscle and so forth (Hammond, 1932).

Focussing on the plane of nutrition, one should take a closer look to the protein:energy ratio. Generally, a higher protein;energy ratio will result in faster growing animals until a certain point (depending on maturity type), as the ratio gets too high then growth may be reduced. Male animals have a higher protein:energy ratio requirement than females. Changing the protein:energy ratio can influence the carcass composition (Campbell & King, 1982).

Animals fed full concentrate diets tend to direct more energy to fat deposition resulting in fatter carcasses than animals that were fed just below ad libitum levels (Irshad *et al*., 2013).

### 2.13.5 Protein

Other than monogastric animals, ruminant animals have the unique ability to break down a soluble protein source to ammonia, where rumen microbes can incorporate the ammonia to produce their own microbial protein. Even higher quality, less soluble protein source, will also be deaminated to a certain extent by the rumen microbes. The higher quality protein may only have differences in the degradation rates. The efficiency of ammonia being incorporated to microbial protein is dependent to the energy content in the diet (Irshad *et al*., 2013).

With ruminants there is a large levelling effect on the protein quality fed to them, hence there is a constant supply of amino acids to the ruminant for muscle growth. As shown in Table 1, rumen microbes' amino acid composition is very close to the animal's lean tissue amino acid composition

Protein quality is less important to ruminant animals, but care should be taken to ensure that the diets still have enough essential amino acids, in the case of cattle, lysine and methionine, play an important role in optimal lean tissue accretion.

The ruminant's age is an important factor to consider when taking protein quality into account as a younger ruminant will need higher quality and higher levels of protein in the diets as the rumen is not so well developed to sufficiently supply adequate amounts of microbial protein.

### 2.13.6 Fat

Ruminant animals can produce their own characteristic fat from the carbohydrate and protein sources in the diet. Fat in moderation can be used as an energy source by the ruminant. The microbes in the rumen hydrogenate unsaturated fat than can be used in milk production in in the accretion of fat tissue (Harfoot & Hazlewood, 1997).

### 2.13.7 B-adrenergic agonists

Chemically produced substances like Zilmax or Grofactor that has zilpaterol hydrochloride as active ingredient to stimulate the β-receptors in certain tissue receptors, acting similar to the effect of epinephrine. B-adrenergic agonists are also seen as effective repartitioning agents (Moloney *et al*., 1991).

Repartitioning agents shift nutrients from fat deposition towards lean tissue accretion. This is effectively utilized in feedlots when animals are nearing maturity and fat deposition starts to increase. With the help of a repartitioning, you can shift wave 3 in Figure 2 A, can be shifted to the right, increasing the lean muscle tissue growth and postponing the fat deposition, allowing for bigger and leaner carcasses that grow more efficiently. The effect of a β-agonist on different species and parameters can be seen in the summary in Table 5. According to Hossner, (2005) β-agonist are most effective when given to older and heavier animals with more body fat.

*Table 5:* Summary of the effects of β-agonists on production characteristics in various farm animals, as percentage from untreated animals (Adapted from Moloney et al., 1991).

| | Species | | | |
|---|---|---|---|---|
| Parameter | Sheep | Cattle | Pigs | Chicken |
| Feed intake | +2 | -5 | -5 | - |
| Feed efficiency | +15 | +15 | +5 | +2 |
| Weight gain | +15 | +10 | +4 | +2 |
| Muscle | +25 | +10 | +4 | +2 |
| Fat | -25 | -30 | -8 | -7 |

### 2.13.8 Environment

Animals are adapted to survive and even thrive in an array of environmental conditions. When choosing animals for a production system, certain breeds are better adapted to certain conditions than other. An animal with the best genetic potential placed in the wrong environment will never reach their full genetic potential as seen for example with Friesland Holsteins in warm humid conditions experiencing heat stress, more energy is directed to cooling and their feed intake patterns may also alter, restricting their energy intake (Baumgard & Rhoads, 2013).

Animals that need to survive in colder environment generally have more hair and generally a more compact frame, whereas animals that needs to survive in warmer conditions generally have less hair, an angular frame with a hump and a dewlap. The thermoneutral zone for breeds of cattle will differ but generally optimal producing temperatures are between 10°C to 20°C. Lighter coloured coats also tend to be better adapted to conditions where radiant energy has a larger role to play, as a lighter coloured coat tend to reflect more light that darker coloured coats. According to Hossner (2005) cattle benefit from longer exposure to light as carcass fat is reduced while carcass protein is increased.

In order to get the best producing cattle in a certain environment, care must be taken in choosing the breed as this can have a marked influence on the feed efficiency and the carcass composition acquired (Irshad *et al*., 2013).

### 2.13.9 Pre-slaughter handling

Stress should be kept to a minimum from last feed consumed to slaughter in order to achieve the highest possible quality carcass. This is a very difficult process to optimize as there is so many variables that can play a role, with stress being the most prominent factor (Irshad *et al*., 2013).

# Chapter 3

# Material and Methods

## 3.1 Selection of cattle for study

A uniform group of 127 cattle was selected out of a group of 350 cattle based on breed (Bonsmara), weight (220kg-280kg) and type (bos taurus). The cattle were selected at Beefcor Feedlot near Bronkhorstspruit.

## 3.2 Experimental design, treatment diets and measured parameters

One hundred and twenty Bonsmara type steers were blocked by body weight (BW) and randomly allocated to four treatments blocked into five blocks (total 20 pens) with six animals per pen in a complete randomised block design experiment. The four treatments were:

1. Basal diet supplemented with urea **(CON)**,
2. Basal diet supplemented with RUP **(RUP)**,
3. Basal diet supplemented with RUP and RPLys and **(RUP+L)** and
4. Basal diet supplemented with RUP and RPLys and RUPMet **(RUP+L+M)**

The ingredient and nutrient composition of the starter, grower and finisher diets are shown in tables 6-8. The diets were formulated using the program AMTS (201 East Cortland street, Groton, NY). All four experimental diets were iso-nutrient in terms of starch, sugar, soluble fiber, fat and fermentable carbohydrate. Treatment 1 (CON) was a commercial diet supplemented with only a RDP source (urea) with RUP (%CP) of 33-36% for the starter, grower and finisher diets respectively, treatment 2 (RUP) was a commercial diet supplemented with RUP sources (primarily Aminomax soya) with RUP(%CP) ranging from 40-43% , treatment 3 (RUP+L) was the same as treatment 2 except that the lysine as a %MP was increased from 6.3% to 6.6% through RPLys supplementation (Ajipro-L, Ajinomoto Heartland) with RUP(%CP) ranging from 42-46% and treatment 4 (RUP+L+M) was the same as treatment 3 except the Lys : Met ratio was increased to 3:1 through supplementation with RPMet (Smartamine M, Adisseo, Antony, France) with RUP (%CP) ranging from 43-46%.

The Urea content of the Con treatment was 0.84% while the urea inclusion in the other treatments varied from 0.12-0.23%. The high RUP soya product (Aminomax) was only included in the RUP, RUP+L and RUP+L+M treatment. The grower and finisher diets contained much higher levels of hominy chop and maize but lower levels of wheat bran compared to the starter diet. When compared to the grower diet most of the wheat bran was replaced with maize in the finisher diet. This resulted in a mean starch level of 29%, 35% and 40% for the starter, grower and finisher diets respectively.

Parameters measured were growth performance, blood and carcass parameters. Performance parameter assessed was average daily gain (ADG), feed conversion ratio (FCR) and dry matter intake per pen (DMI). Blood parameters measured were blood urea nitrogen (BUN), creatinine and serum AA profile. Carcass parameters assessed were last live weight, cold carcass mass, warm carcass mass, dressing percentage, liver weight, compactness, fat thickness on the 13$^{th}$ rib and channel fat area.

Table 6: Ingredient and nutrient composition of the starter diets (%DM)

| **Ingredient(%DM)** | **CON** | **RUP** | **RUP+L** | **RUP+L+M** |
|---|---|---|---|---|
| Hominy chop | 39.3 | 37.4 | 37.4 | 37.4 |
| Dried Brewers grain | 8.26 | 2.57 | 4.37 | 4.50 |
| Wheat bran | 24.7 | 25.5 | 24.3 | 24.3 |
| Maize crushed coarse | 5.23 | 6.32 | 6.98 | 6.98 |
| Erragrostis curvula hay | 6.45 | 7.92 | 7.90 | 7.51 |
| Molasses cane | 14.1 | 12.0 | 12.7 | 12.7 |
| AminoMax Soya[1] | 0.00 | 5.34 | 3.95 | 4.04 |
| Sunflower oilcake meal | 0.000 | 0.933 | 0.690 | 0.706 |
| Gluten 20 | 0.000 | 0.709 | 0.525 | 0.537 |
| Urea | 0.782 | 0.225 | 0.111 | 0.111 |
| Premix | 0.067 | 0.067 | 0.067 | 0.067 |
| Limestone | 1.095 | 0.989 | 0.962 | 1.014 |
| AjiPro L[2] | 0.000 | 0.000 | 0.111 | 0.111 |
| Smartamine M[3] | 0.000 | 0.000 | 0.000 | 0.034 |
| **Nutrient composition (%DM)** | | | | |
| CP | 15.03 | 15.09 | 14.23 | 14.33 |
| RUP (%CP) | 33.53 | 42.54 | 42.42 | 42.59 |
| ME (MJ/kg) | 10.52 | 10.69 | 10.73 | 10.73 |
| NFC | 42.93 | 42.96 | 43.26 | 43.28 |
| Starch | 28.74 | 28.85 | 29.04 | 29.05 |
| NDF | 28.4 | 29.0 | 28.8 | 28.7 |
| Ca | 0.7 | 0.64 | 0.64 | 0.66 |
| P | 0.53 | 0.56 | 0.54 | 0.54 |
| Lys (%MP) | 6.3 | 6.33 | 6.63 | 6.6 |
| Met (%MP) | 2.25 | 2.12 | 2.15 | 2.36 |

CP=crude protein; RUP = rumen undegradable protein; ME = metabolisable energy; NFC = non-fibrous carbohydrate; NDF = neutral detergent fiber; Ca = calcium; P = phosphorous; Lys = lysine; Met = methionine; MP = metabolisable protein

[1]High RUP heat treated soya; [2]Rumen protected lysine; [3]Rumen protected methionine

Table 7:Ingredient and nutrient composition of the grower diets (%DM)

| **Ingredient (%DM)** | **CON** | **RUP** | **RUP+L** | **RUP+L+M** |
|---|---|---|---|---|
| Hominy chop | 50.3 | 50.3 | 50.3 | 50.3 |
| Dried brewers grain | 5.77 | 3.63 | 2.23 | 2.23 |
| Wheat bran | 10.6 | 10.1 | 9.4 | 9.4 |
| Maize crushed coarse | 12.3 | 12.3 | 12.3 | 12.3 |
| Eragrostis curvula hay | 6.48 | 7.06 | 7.53 | 7.53 |
| Molasses cane | 12.3 | 11.3 | 11.1 | 11.1 |
| AminoMax Soya | 0.00 | 2.98 | 4.36 | 4.30 |
| Sunflower oilcake meal | 0.000 | 0.521 | 0.761 | 0.752 |
| Gluten 20 | 0.000 | 0.396 | 0.579 | 0.571 |
| Urea | 0.866 | 0.247 | 0.124 | 0.123 |
| Premix | 0.074 | 0.074 | 0.075 | 0.074 |
| Limestone | 1.211 | 1.085 | 1.065 | 1.122 |
| AjiPro L v3 | 0.000 | 0.000 | 0.124 | 0.123 |
| Smartamine M | 0.000 | 0.000 | 0.000 | 0.037 |
| **Nutrient composition(%DM)** | | | | |
| CP | 13.88 | 13.04 | 13.11 | 13.1 |
| RUP (%CP) | 36.07 | 43.17 | 48.57 | 45.91 |
| ME (MJ/kg) | 10.88 | 11.11 | 11.17 | 11.17 |
| NFC | 48.05 | 47.98 | 48.02 | 47.99 |
| Starch | 35.48 | 35.39 | 35.25 | 35.24 |
| NDF | 23.7 | 24.0 | 24.0 | 24.0 |
| Ca | 0.71 | 0.66 | 0.65 | 0.67 |
| P | 0.45 | 0.46 | 0.46 | 0.46 |
| Lys (%MP) | 6.19 | 6.26 | 6.61 | 6.6 |
| Met (%MP) | 2.26 | 2.19 | 2.15 | 2.4 |

CP=crude protein; RUP = rumen undegradable protein; ME = metabolizable energy; NFC = non-fibrous carbohydrate; NDF = neutral detergent fiber; Ca = calcium; P = phosphorous; Lys = lysine; Met = methionine; MP = metabolizable protein
[1]High RUP heat treated soya; [2]Rumen protected lysine; [3]Rumen protected methionine

Table 8: Ingredient and nutrient composition of the finisher diets (%DM)

| **Ingredient(%DM)** | **CON** | **RUP** | **RUP+L** | **RUP+L+M** |
|---|---|---|---|---|
| Hominy chop | 53.1 | 53.1 | 52.4 | 52.4 |
| Dried brewers grain | 4.33 | 3.46 | 0.00 | 0.00 |
| Wheat bran | 0.92 | 1.34 | 2.07 | 2.09 |
| Maize crushed coarse | 19.7 | 19.8 | 19.7 | 19.8 |
| Erragrostis curvula hay | 8.71 | 8.74 | 9.45 | 9.44 |
| Molasses cane | 10.6 | 10.0 | 9.8 | 9.6 |
| AminoMax Soya | 0.00 | 1.25 | 3.68 | 3.71 |
| Sunflower oilcake meal | 0.000 | 0.232 | 0.642 | 0.648 |
| Gluten 20 | 0.369 | 0.534 | 0.857 | 0.862 |
| Urea | 0.894 | 0.256 | 0.128 | 0.127 |
| Premix | 0.077 | 0.077 | 0.077 | 0.077 |
| Limestone | 1.252 | 1.127 | 1.102 | 1.162 |
| AjiPro L v3 | 0.000 | 0.000 | 0.128 | 0.127 |
| Smartamine M | 0.000 | 0.000 | 0.000 | 0.038 |
| Zilmax | 0.012 | 0.012 | 0.012 | 0.012 |
| **Nutrient composition(%DM)** | | | | |
| CP | 12.96 | 11.49 | 11.86 | 11.9 |
| RUP (%CP) | 33.93 | 40.48 | 44.33 | 44.49 |
| ME (MJ/kg) | 11.37 | 11.53 | 11.6 | 11.59 |
| NFC | 51 | 51.7 | 51.91 | 51.79 |
| Starch | 39.79 | 40.53 | 40.37 | 40.38 |
| NDF | 21.7 | 22.0 | 22.1 | 22.1 |
| Ca | 0.7 | 0.64 | 0.63 | 0.65 |
| P | 0.38 | 0.4 | 0.41 | 0.41 |
| Lys (%MP) | 6.39 | 6.36 | 6.75 | 6.73 |
| Met (%MP) | 2.33 | 2.29 | 2.21 | 2.47 |

CP=crude protein; RUP = rumen undegradable protein; ME = metabolizable energy; NFC = non-fibrous carbohydrate; NDF = neutral detergent fiber; Ca = calcium; P = phosphorous; Lys = lysine; Met = methionine; MP = metabolizable protein
[1]High RUP heat treated soya; [2]Rumen protected lysine; [3]Rumen protected methionine

## 3.3 Processing of animals

All the cattle were processed at Beefcor the day before transportation to UP experimental farm in Hatfield. The cattle came from backgrounding and were administered Bovishield Gold (respiratory BRD vaccine Infectious bovine rhinotracheitis. parainfluenza 3. bovine repertory syncytial virus. Both BVD's). One shot ultra (all clostridial). Botsure (botulism). Lumpyvax (lumpy skin). Reverin / Tennaline (tetracycline antibiotic for metafalaxis). Revalor H (Implant). Ivermax (ivermectic. dewormer and anti-tick) and Ivomec super.

The cattle had a 5-digit ear tag in the left ear that was used as ID for each animal. During the sorting of cattle at UP experimental farm, a day after arrival. the cattle were given an ear tag based on their treatment group. Each treatment had a colour ear tag assigned to the treatment.

- Control = red
- RUP = blue
- RUP+L = Green
- RUP+L+M = Yellow

## 3.4 Daily management of feedlot

Water was available ad lib and cattle were fed twice daily (06h30 and 16h00). During the morning feeding, feed bunk scoring was done whereafter orts were weighed back for each pen. The cattle were fed 60% of their daily feed allocation in the morning and 40% in the afternoon. Before feeding 7% water was added to the feed as no water was added during mixing of the feed to extend shelf life. Feeding was adjusted daily to prevent over or underfeeding. If the orts weighed less than 1kg, feed per pen was increased by 0.25kg/head and if the orts weighed more than 2kg the feed was decreased by 0.25kg/head. If the pen had less than 1kg of orts for two consecutive days, the feed was increased by 0.5kg/head. After feeding, the cattle were observed for any signs of illness and manure scoring was performed. Manure scoring is sound biological basis for evaluating the efficiency of healthy rumen function and extent of feed digestibility (Hulsen, 2005) A scale of 1-5 was used, where a score of 1 (Figure 3) is very loose watery manure that isn't ideal and indicative of an unhealthy rumen environment and potential acidosis. Manure score 3 (Figure 4) is the ideal manure and has a thick custard like consistency with concentric rings and typically forms a small depression in the centre. Manure score 5 is manure that is too firm with balls stacking 5-10cm high, indicative of too much roughage and potentially dehydration. If a pen had a score of 1 for two consecutive days, then 0.25kg/head of milled hay was added to the pen to assist with rumen health. As soon as the manure score was back to 3 then the hay supplementation was terminated.

Figure 3:Manure score of 1 for feedlot cattle. indicating an unhealthy rumen

Figure 4: Manure score of 3 for feedlot cattle. Ideal manure for feedlot cattle

During the first six days of the trial the cattle were adapted to the new environment. Cattle were fed ad lib hay and 6kg/head of starter per day, decreasing the hay each day to stimulate starter intake.

At the onset of the starter phase the cattle were fed at 8-10 kg/head, whereafter the feed was adjusted based on the DMI. Moving from starter to finisher the starch and NFC content of the diets increased. The starter was fed for 21 days and on day 22 the grower phase was implemented with a 3 day step up period where the starter to grower ratio was changed from 2/3 starter: 1/3 grower, ½ starter: ½ grower and 1/3 starter: 2/3 grower. The grower was fed

for a total of 74 days whereafter animals were adapted to the finisher diets for 2 days by feeding finisher in the mornings and grower in the afternoon. The finisher was fed for 40 days with a grofactor withdrawl period of 5 days. The total feedlot growth trial was 135 days.

The active ingredient in Grofactor is zilpaterol hydrochloride. Grofactor is classified as a beta-agonist. that are known as a repatitioning agent. A repartitioning agent directs more growth to muscle accretion and away from adipose tissue growth. resulting in more efficient growth as it requires less energy to grow a unit of muscle than a unit of fat. Grofactor was used in the finisher phase as the cattle is generally closer to maturity, when fat deposition starts to increase as seen in Figure 2. The repartitioning agent then directs more energy towards muscle growth than to fat growth. extending curve 3 (Figure 2) a few days longer. promoting growth performance and carcass characteristics. Carcasses are heavier and leaner when a repartitioning agent is fed. (Hosford *et al*., 2015)

## 3.5 Weighing and blood sampling

Cattle were weighed a day after arrival at the feedlot as well as at the end of each feeding phase and every 14-21 days between phases. Body condition scoring was done during each weighing.

Three cattle were randomly selected from each pen for blood samples. Blood samples were collected at the onset of the trial and at the end at each feeding phase. Blood was sampled from the coccygeal tail vein using a red-top vacutainer, where after the ID of the animal and time of collection was recorded on the vacutainer. The blood was allowed to clot for 30 minutes whereafter it was centrifuged at 4000 rpm for 8 minutes. The serum was aliquoted into 2 samples and transferred to Eppendorf tubes. One sample was sent to Clinical Pathology at Onderstepoort for BUN and creatinine analyses and the other sample was sent to NWU Potchefstroom for AA-profile analyses.

## 3.6 Sample analyses

### 3.6.1 Feed samples

There was a total of eighteen feed batches and a subsample taken from each batch. Samples were taken at 4 random spots from each batch in order to get a representative sample. Samples were stored in a freezer at -18 ˚C. Samples from each phase of the trial was pooled together and a representative sample of that phase was sent to Nutrilabs for analysis. Samples were analysed for the following nutrients:

- DM (Dry matter) (AOAC.200 procedure 934.01)
- ASH (AOAC. 200) (AOAC.200 procedure 942.05)

- CP (Crude protein) (Leco analyser) (AOAC.200 procedure 968.06)
- NDF (Neutral detergent fibre) (ANKOM Technology Method 9)
- ADF (Acid detergent fibre) (ANKOM Technology Method 8)
- EE (AOAC. 200 procedure 920.39. using Soxtec 2043)
- ADIN (Goering *et al*., 1970)
- Ca (Calcium) (AOAC. 200)
- P (Phosphorus) (AOAC. 200)
- NFC (Non fiber carbohydrate) (Calculated according to NRC. 2001)
    - NFC = [100-(%NDF+%CP+%EE+%Ash)]

Orts were sampled once per week. All the samples collected were pooled within treatments. Samples were only taken when the orts were more than one kilogram. The orts were analysed for:

- DM (Dry matter) (AOAC.200 procedure 934.01)
- NDF (Neutral detergent fibre) (ANKOM Technology Method 9)
- CP (Crude protein) (Leco analyser) (AOAC.200 procedure 968.06)

### 3.6.2 Blood samples: amino acid profile

The amino acid profile evaluation was performed at NWU Pothefstroom, using an Agilent HP 7890A gas chromatograph (GC) (Hewlett-Packard Company. North Carolina. USA) coupled to an Agilent 5975C MSD with Triple-Axis Detector (Aligent Technologies. California. USA).

Amino acid extracts (1 uL) were analysed in splitless mode. A GC column (Zebron ZB-AAA. 10 m x 0.25 mm), which is included in the analyses kit, was used for compound separation, with ultra-pure helium as the carrier gas at a constant flow of 1.3 mL/min and pressure of 0.37 psi. The inlet temperature was kept constant at 250°C throughout the run. The oven temperature was initially held at 60°C for 1 min, where after it was increased by 50°C/min to 110°C. then with 20°C/min to 185°C. followed by an increase of 25°C/min to 235°C. and finally with 30°C/min to an ending temperature of 320°C. where it was it was kept for 1 min.

Peak detection. integration and quantification were done using Enhanced MSD Chemstation software (Agilent Technologies. Inc., USA. version F.01.00.1903). The data was

quantified by comparing the intensities of the detected compounds to that of the corresponding internal standards. ERNDIM IQCS amino acids levels 1 and 2 (MCA Laboratory. The Netherlands) were used for quality control purposes.

### 3.7 Carcass evaluation

The cattle were slaughtered at Chamdor abattoir in Krugersdorp. Seven cattle were selected at Beefcor during the selection of the group of 127 cattle. The seven cattle remained at Beefcor and was fed 4kg starter of Beefcor's ration with ad lib hay until they were slaughtered the next week at Chamdor abattoir. The 7 initially slaughtered cattle provided a baseline for carcass evaluation of the diets and at the end of the 135-day feeding period the cattle were slaughtered. Final bodyweights were determined a day before slaughter at the feedlot. As the cattle entered the abbattoir, the treatment colour and ID were recorded.

During the evisceration rumen scoring was done by Prof Leon Prozesky (5 Olive tree ave, The hills estate, Garsfontein, Pretoria). Rumens were marked via a coloured tie strap according to the slaughter order. After the rumens were marked, they were moved to the offal room for scoring. In the offal room the rumens were opened, and the contents removed to observe if any damage was present on the rumen wall. The scoring was done by as follows: Normal = All surfaces of the rumen appear healthy with long and thick papillae, Mild = Focal. focal extensive areas of sparse. short. denuded papillae, Moderate = Focal extensive areas of sparse. short. denuded papillae associated with congestion/ haemorrhage and necrosis, Severe = Focal. multifocal lesions/ scars. Each animals' liver was weighed and recorded using a scale. Hot carcass mass was determined 45 minutes after evisceration.

After the carcasses were chilled for 24 hours the following measurements were obtained: compactness, cold carcass mass, channel fat mass, fat thickness between the 12$^{th}$ and 13$^{th}$ rib and the dressing percentage. The compactness was determined by measuring the length of the carcass from the acromion on the scapula until the symphysis of the pubis divided by the cold carcass mass. The cold carcass mass was determined by placing the carcasses on a scale after the 24-hour period. Channel fat mass was determined by measuring the longest, widest and deepest point of the channel fat to determine a volume (V=LxBxH). The volume of fat measured was converted to grams using a conversion factor. The conversion factor was determined by using a sample of the channel fat. The sample was weighed and placed in a volumetric cylinder to determine how much volume is displaced. Twenty-two grams of fat displaced 20.24ml of water in the volumetric flask. Meaning 1g of fat displaces 0.92ml of volume. hence the conversion factor being 0.92. the fat thickness between the 12$^{th}$ and 13$^{th}$ rib was taken 50mm from the median of the carcass using a vinier.

## 3.8 Animal mortalities and morbidities

After the morning feeding. the animals were observed for any signs of illness or discomfort. When a sick animal was identified. it was taken to the crush where it was observed and examined. The ID was recorded along with body temperature and any other obvious problems. Thereafter the animal was treated accordingly. The animal was then moved to a sick pen and fed the same treatment diet. Sick animals were moved back to their pen if the DMI intake was the same as the mean pen intake for 2 consecutive days.

## 3.9 Statistical analysis

Data were analyzed statistically with the Proc Mixed model (Statistical Analysis System. 2021) for mean effects. Means and standard error were calculated and significance of difference ($P<0.05$) between means were determined by Fischers test (Samuels., 1989). Repeated Measures Analysis of Variance with the mixed model were used for repeated period measures. Outliers was excluded from results by using two standard deviations from the means.

The linear mix model used is described by the following equation:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}$$

Where $Y_{ij}$ = variable studied during the period

$\mu$ = overall mean of the population

$T_i$ = effect of the $i^{th}$ treatment

$B_j$ = effect of the $j^{th}$ block

$e_{ij}$ = error associated with each Y

## 3.10 Animal Ethics clearance

Before the trial commenced the trial was waved for sec 20 approval and the animal ethic committee approved the trial with reference number NAS047/2020

# Chapter 4

# Results and Discussion

## 4.1. Feed analyses

The nutrient composition of the starter, grower and finisher diets are shown in Tables 9 to 11. The mean CP% was 13.0, 11.2 and 10.4% for the starter, grower, and finisher diets respectively and the mean NFC% was 43.8, 47, 49.4% respectively. This is in agreement with nutrient guidelines used by feedlot nutritionists in practical feedlot formulation (NASEM,2016; Vermaak, 2019) Differences in theoretical nutrient profiles of the diet formulations in Tables 6-8 and the actual nutrient analyses are expected since the AMTS formulation program databases were used for theoretical formulations. It must also be noted that although the best efforts were done to get a representative sample, it still leave some room for variation and error when a sample is taken.

Table 9: Nutrient composition of the experimental starter diets (%DM)

| Nutrient | Unit | CON | RUP | RUP+L | RUP+L+M |
|---|---|---|---|---|---|
| DM | % | 77.5 | 77.6 | 76.6 | 77.7 |
| CP | % | 12.8 | 13.2 | 12.9 | 13.1 |
| NDF | % | 33.2 | 33.2 | 33.7 | 33.9 |
| ADF | % | 12.6 | 12.2 | 12.9 | 12.5 |
| EE | % | 4.42 | 4.75 | 4.40 | 4.57 |
| Ash | % | 5.28 | 5.20 | 5.18 | 5.13 |
| Ca | % | 0.99 | 1.02 | 0.90 | 1.07 |
| P | % | 0.62 | 0.57 | 0.61 | 0.54 |
| ADIN | % | 5.79 | 5.87 | 5.51 | 5.37 |
| NFC | % | 44.3 | 43.7 | 43.8 | 43.4 |

DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; Ca = calcium; P = phosphorus; ADIN = acid detergent insoluble nitrogen; NFC = non-fibrous carbohydrate

Table 10: Nutrient composition of the experimental grower diets (%DM)

| Nutrient | Unit | CON | RUP | RUP+L | RUP+L+M |
|---|---|---|---|---|---|
| DM | % | 77.2 | 77.0 | 76.7 | 76.7 |
| CP | % | 10.7 | 11.8 | 10.8 | 11.5 |
| NDF | % | 33.7 | 32.8 | 30.6 | 30.5 |
| ADF | % | 12.8 | 12.5 | 11.4 | 11.7 |
| EE | % | 5.52 | 5.06 | 5.24 | 5.32 |
| Ash | % | 4.60 | 4.68 | 4.58 | 4.93 |
| Ca | % | 0.90 | 1.24 | 1.07 | 1.04 |
| P | % | 0.67 | 0.58 | 0.52 | 0.49 |
| ADIN | % | 4.54 | 5.07 | 5.02 | 5.70 |
| NFC | % | 45.5 | 45.7 | 48.8 | 47.8 |

DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; Ca = calcium; P = phosphorus; ADIN = acid detergent insoluble nitrogen; NFC = non-fibrous carbohydrate

Table 11: nutrient composition of the experimental finisher diets (%DM)

| Nutrient | Unit | CON | RUP | RUP+L | RUP+L+M |
|---|---|---|---|---|---|
| DM | % | 75.7 | 75.8 | 76.3 | 75.7 |
| CP | % | 9.44 | 11.5 | 10.1 | 10.4 |
| NDF | % | 29.1 | 30.3 | 30.7 | 27.2 |
| ADF | % | 11.1 | 11.1 | 11.3 | 9.90 |
| EE | % | 5.67 | 5.57 | 6.95 | 6.77 |
| Ash | % | 4.58 | 4.63 | 4.48 | 4.90 |
| Ca | % | 1.15 | 1.10 | 1.10 | 1.13 |
| P | % | 0.47 | 0.48 | 0.46 | 0.48 |
| ADIN | % | 4.96 | 5.28 | 5.23 | 5.15 |
| NFC | % | 51.2 | 48.0 | 47.8 | 50.8 |

DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; Ca = calcium; P = phosphorus; ADIN = acid detergent insoluble nitrogen; NFC = non-fibrous carbohydrate

## 5.1. DMI and growth performance

In Table 12 is shown the mean BW, ADG, FCR and DMI during the different growth periods as well as the overall growth period of 135 days. The mean starting weight was 250.8kg with less than 1kg difference between the starting weights of the four treatment groups reflecting highly uniform starting groups. According to Olivier (2021) the norm for cattle entering large commercial feedlots in South Africa is 253kg with a slaughter mass of 465kg and days on feed (DOF) of 135. This is different from the USA where cattle enter and exit the feedlot at heavier weights and with longer DOF. In a recent nutritionist survey that involved 14 million USA feedlot cattle, 71% of receiving calves weighed between 272kg and 363kg with 91% of the cattle reaching slaughter weights ranging from 590 kg to 680kg with mean DOF of 201 days (Sameulson *et al*., 2016). It is therefore not always possible to directly compare growth performance of cattle in USA feedlot trails with South African studies.

The mean slaughtering mass was 501kg and varied between 498kg for the RUP treatment and 507kg for the CON treatment and did not differ between treatments (P>0.05).

Haasbroek (2013) conducted a feedlot growth trail at a large commercial feedlot involving 900 cattle and reported a mean starting weight of 225kg and mean slaughtering weight of 425kg. The DOF was only 115 days. Miles (2015) used 144 Bonsmara type steers that were fed a hominy chop-based diet and reported a mean starting weight of 233kg and mean slaughter weight of 461kg. The current trend, however, is to use cattle with a slightly higher starting around 250kg compared to 5 years ago (Olivier, 2021).

The ADG did not differ between treatments and varied from 1.84kg/d to 1.91 kg/d over the total 135 day feeding period (P>0.05). Niemand (2013) performed a meta-analyses using historical growth data from South African feedlots and compared the growth performance of 48 600 Drakensberger cattle with 449 198 head from other breeds and crossbreds. The mean ADG was 1.65 and 1.67 kg/d for the Drakensberger and other cattle respectively. The mean ADG from three other South African commercial feedlot trails were 1.65 kg/d (Steenkamp, 2014), 1.73 kg/d (Haasbroek, 2013) and 1.76 kg/d (Miles,2015). The animals in our study were in small pens and therefore under less stress than commercial feedlot cattle which explains the somewhat better performance. The study by Haasbroek (2013) also included a small pen study with 180 Bonsmara type cattle and reported a ADG of 1.82 to 1.89 kg/d which is in agreement with our results. It is also evident that growth performance of feedlot cattle has improved over the years, since production statistics of 11 feedlots from 1982-1988 showed an ADG of only 1.23kg/d (De Bruyn, 1991). In earlier studies Kreiner *et al* (1991) reported a ADG of 1.35 kg/d for Brahman and Drakensberger Bulls and Chipa *et al* (2010) found a ADG of 1.5kg/d with Nguni bulls fed diets containing different levels of cold pressed soybean oilcake.

The mean FCR varied between 4.84 and 5.04 kg feed/kg gain and did not differ between treatments (P>0.05). Similar results were reported by Leeuw *et al* (2009) and Miles (2015) when Bonsmara type cattle were fed maize / hominy chop-based diets. Haasbroek (2013) found FCR of 4.5-4.9 kg feed/kg gain and 5.2-5.6 kg feed/kg gain for the small pen study and commercial pen studies respectively which is in agreement with our data.

The experimental treatments differed in mean CP content from 13.0% for the starter to 10.6% for the grower diet and the RUP content varied from 34-44%. Treatments differed in protein quality with the supplementary protein sources being either urea, a soybean rumen protected source with 65% RUP (AminoMax) and AminoMax supplemented with RPLys or RPLys+Met. The current NRC recommendation for cattle of similar weight and ADG varies from 17.2% CP in the starter phase to 11% CP in the finisher phase. The corresponding RUP recommendations are 50% and 33% respectively (NASEM, 2016). Our formulations broadly falls within these recommendations but with lower starting diet CP of only 13%. The rational for the high initial CP and RUP is the fact that young weaners have not been exposed to

concentrates and therefore have an initial depressed microbial protein synthesis capacity and low DMI that has to be supplemented with higher levels of RUP in order to meet requirements (Zinn, 2014). Meissner *et al* (1992) recommended CP levels of 10-14% and RUP levels of 35-40% for South African feedlots during the three growth phases. Chester-Jones & DiCostanzo (2012) recommended 12.5 to 14.5% CP for newly received calves. Evidence from a number of USA feedlot studies suggested that finishing cattle have a protein requirement of 12-13% and feeding any concentration greater than that seems to have no advantage (Bailey & Duff, 2005)

Deficiencies in essential AA in the early growing phase have shown a negative influence on ADG and DMI (Hussein & Berger, 1995; Wessels *et al.,* 1997). Research on the AA requirements of growing cattle states that Met is the primary limiting AA with Lys the second. With few exceptions (i.e. fishmeal) protein supplements commonly fed to feedlot cattle (oilseed meals, maize by-products) are not good sources of Met and /or are deficient in Lys (Wessels *et al.,* 1997). There is therefore renewed interest in the role of RPAA in the nutrition of feedlot cattle during the early growth phases (Torrentera *et al.,* 2016; Prestegaard *et al.,* 2017). Compared to dairy cattle limited research has been published on the supplementation of RPAA to feedlot cattle. In some studies, RPAA (Lys and Met) improved performance (Hosford et al., 2015; Torrentera *et al.,* 2016; Prestegaard *et al.,* 2017) but in others no responses were found (Oney *et al.,* 2016; Heiderscheit & Hansen, 2020).

Zinn (2014) recommended that feedlot diets should be formulated to provide a minimum of 2.1 and 7.0 g/kg dietary DM intake of metabolizable lysine and methionine respectively, therefore a ratio of 3.3:1 as also recommended by Li *et al* (2019). Our diets were formulated using AMTS and the starter and grower diets varied in Lys:Met ratio from 2.8:1 to 3.1:1 which is similar to above mentioned recommendations. The Lys and Met expressed as % of requirements more than 100% except for the control treatment with a 90% of requirement prediction. A higher actual than model predicted DMI would result in all the treatments supplying Lys and Met above requirements which could have contributed to the lack of treatment response in growth performance. The lack of response in performance in our study was unexpected, based on the published research results as discussed above. A combination of factors could have contributed to this.

Firstly, the weaners used in our study have been backgrounded and therefore have been exposed to concentrates which contribute to rumen papillae development and the establishment of a rumen microbial population (Diao *et al*., 2019). Weaners that have not been exposed to concentrates take much longer to reach an acceptable DMI and to establish a robust microbial population. In our study the mean DMI on day 26 was already 2.7% of BW

indicating a well-developed rumen and potentially satisfactory microbial protein production. Under such conditions supplemented RUP might be of lesser importance.

Secondly the control diet contained 50.3% hominy chop, 12.3% maize and 5.8% brewers grain. The RUP content of these feedstuffs is 32%, 47% and 77% of CP respectively (Erasmus *et al*., 1988,1990). It is therefore possible that the RUP and AA contribution from these feeds, together with relatively high DMI caused RUP not to be a limiting factor. Because the RUP content of maize and maize by-products is relatively high, MP supply in most maize based diets is close to or exceeds the requirement of feedlot cattle, especially later in the feeding period (DiCostanzo, 2007). Miles (2015) formulated diets with high levels of maize and maize by-products, including up to 0.9% urea with only 2% soybean meal and achieving a mean ADG of 1.76 kg/d and a mean FCR of 4.8 supporting our argument that RUP might not be limiting on these diets. A summary of 10 trails conducted by Martin *et al* (1980) at Oklahoma state University comparing urea and soybean meal found that animals fed urea were superior to those fed soybean meal in terms of ADG (5.0%) and FCR (1.1%) when whole shelled dry rolled, or steam flaked maize based diets were fed. An interesting observation was the reduction in feed intake of the cattle in our study fed the RUP+L+M treatment. DMI intake was lower overall when compared to CON but also lower when compared to treatment RUP from day 68-99 and day 100-134. A meta-analysis by Patton (2010) found that RPMet slightly decreased DMI. In contrast supplementation of cracked maize-based diets fed to steers with rumen protected soybean meal instead of urea improved ADG and FCR but only for the first 28 days on feed (Ludden *et al*., 1995). It is possible that the cracked maize decreased the starch rate of fermentation and thereby depressing microbial protein synthesis resulting in steers being more responsive to an increased supply of RUP.

A Third factor that might have impacted our results is the starting weight of the weaners. The mean starting weight was 250.6kg with some weaners weighing up to 280kg at the onset of the trail. Studies by Ludden *et al* (1995), Sindt *et al* (1995) and Klemesrud *et al* (2000) found that supplementing diets with high RUP protein sources to meet MP requirements had little or no effect on performance once cattle reached a BW of more than 310kg. In the USA it is common to feed Holstein calves that are procured at BW of 120-150kg. It is recommended that nutritionist must consider the RUP content and the AA profile of bacterial and RUP AA only for the period when calves weigh 136-318kg (DiCostanzo, 2007). Torrentera *et al* (2016) supplemented 150 holstein steer calves with mean BW of 127kg with lysine and methionine and found increased FCR for the first 56 days of the trail. It can therefore be speculated that a lower starting weight of 200-220kg would have been more applicable for the hypothesis tested in this trail.

Table 12: The effect of supplemented Lysine. Methionine and Urea on the growth performance of beef cattle

| Item | CON | RUP | RUP+L | RUP+L+M | SE |
|---|---|---|---|---|---|
| **Body weight (kg)** | | | | | |
| Day 1 | 251.2 | 250.6 | 252.4 | 250.7 | 5.76 |
| Day 26 | 311.5 | 309.3 | 311.5 | 313.27 | 7.00 |
| Day 47 | 348.4 | 347.3 | 350.4 | 349.3 | 6.57 |
| Day 67 | 381.9 | 381.7 | 385.7 | 384.5 | 6.67 |
| Day 99 | 440.9 | 439.1 | 440.5 | 439.7 | 6.66 |
| Day 134 | 507.0 | 497.7 | 502.7 | 502.5 | 6.09 |
| **ADG (kg/d)** | | | | | |
| Day 1-25 | 2.32 | 2.26 | 2.27 | 2.41 | 0.09 |
| Day 26-47 | 1.68 | 1.72 | 1.77 | 1.64 | 0.11 |
| Day 48-67 | $1.68_{x}$ | $1.72_{xy}$ | $1.77_{y}$ | $1.76_{xy}$ | 0.04 |
| Day 68-99 | 1.85 | 1.79 | 1.71 | 1.72 | 0.05 |
| Day 100-134 | $1.89_{a}$ | $1.68_{b}$ | $1.78_{ab}$ | $1.79_{ab}$ | 0.05 |
| Day 1-134 | 1.90 | 1.86 | 1.90 | 1.84 | 0.04 |
| **FCR (kg/kg)** | | | | | |
| Day 0-25 | 3.70 | 3.73 | 3.64 | 3.57 | 0.15 |
| Day 26-47 | 5.70 | 5.52 | 5.29 | 6.03 | 0.34 |
| Day 48-67 | $5.86_{x}$ | $5.33_{y}$ | $5.37_{xy}$ | $5.68_{xy}$ | 0.20 |
| Day 68-99 | 5.30 | 5.40 | 5.47 | 5.12 | 0.19 |
| Day 100-134 | $5.37_{ab}$ | $5.65_{a}$ | $5.34_{ab}$ | $4.95_{b}$ | 0.18 |
| Day 1-134 | 5.04 | 4.98 | 4.84 | 4.95 | 0.12 |
| **FI/day (kg/animal/day)** | | | | | |
| Day 1-25 | 8.56 | 8.42 | 8.10 | 8.58 | 0.21 |
| Day 26-47 | 9.53 | 9.32 | 9.33 | 9.60 | 0.28 |
| Day 48-67 | $9.81_{ab}$ | $9.13_{a}$ | $9.49_{ab}$ | $9.98_{b}$ | 0.23 |
| Day 68-99 | $9.76_{a}$ | $9.68_{a}$ | $9.35_{ab}$ | $8.78_{b}$ | 0.28 |
| Day 100-134 | $10.12_{a}$ | $9.46_{b}$ | $9.42_{b}$ | $8.85_{c}$ | 0.16 |
| Day 1-134 | $9.59_{a}$ | $9.24_{ab}$ | $9.14_{ab}$ | $9.07_{b}$ | 0.15 |
| **Days on feed** | 135 | 135 | 135 | 135 | |

a.b.c = $p<0.05$; x.y.z = $p<0.1$; SE = Standard error; ADG = Average daily gain; FCR = Feed conversion ratio; FI = Feed intake

## 5.2. Blood serum parameters

Monitoring blood urea N (BUN) or milk urea N (MUN) can be used as a diagnostic tool for measuring protein and energy status as well as efficiency of N utilisation in cattle (Hammond, 1983). For growing steers BUN levels of between 11 and 15 mg/dL were associated with maximum ADG. With finishing steers BUN values of 7-8 mg/dL resulted in best performance (Preston *et al*., 1978). The effect of dietary treatment on BUN is shown in Table 13. Samples taken on day 26 and 99 were lower in BUN for the RUP+L and RUP+L+M treatments when compared to the CON and RUP diets ($P<0.05$). The CON diet contained the highest level of urea and as expected resulted in the highest BUN levels since BUN and rumen $NH_3$ concentration is highly correlated (Hennessy & Nolan, 1988). Similar results were reported in recent studies where rumen protected AA were supplemented to feedlot steers (Teixeira *et al*., 2019; Heiderscheidt & Hansen, 2020). Reduced BUN levels for the RUP+L and RUP+L+M treatments on day 26 ($P<0.05$) but not on day 134 suggest that the RPAA was effectively used for tissue growth early in the growth phase and is in agreement with findings by Texeira *et al* (2019). Batista *et al* (2016) supplemented steers with 15 Lys/kg and also reported lower SUN concentrations. Lower SUN values in steers supplemented with RP-Lys furthermore suggest that other AA in circulation were also more effectively used for protein tissue deposition rather than excreted as urea (Texeira *et al*., 2019).

Creatinine level in blood is an indication of protein turnover in muscle tissue (Kaneko *et al*., 2008) and the effect of treatment on blood serum creatinine concentration in our study is shown in Table 13. In terms of creatinine concentrations, a general trend observed in all experimental groups was moderately high concentrations of creatinine on d 1, probably due to greater muscle protein metabolism for gluconeogenesis caused by the transport of animals and lower DMI before the feeding of treatments commenced. As the intensive feeding commenced the protein turnover increased from d 26 to d 134. High concentrations of blood creatinine were observed in treatments CON and RUP+L+M compared to the RUP and RUP+L treatments which suggest low muscle protein turnover in the latter 2 treatments and lower muscle gain. However, at d 134 the difference in creatinine concentration between treatment groups was negligible. These results indicate some sensitivity of feedlot to the quality of dietary protein on muscle protein turnover, but more research is required to elucidate this aspect further

Table 13: The effect of supplemented Lysine. Methionine and Urea on BUN and Creatinine

| Item | CON | RUP | RUP+L | RUP+L+M | SE |
|---|---|---|---|---|---|
| **BUN (mg/Dl)** | | | | | |
| Day 0 | $8.24_{a}$ | $7.35_{ab}$ | $6.90_{b}$ | $7.55_{ab}$ | 0.438 |
| Day 26 | $9.52_{a}$ | $9.62_{a}$ | $7.28_{b}$ | $6.72_{b}$ | 0.466 |
| Day 99 | $9.45_{a}$ | $8.55_{ab}$ | $6.76_{bc}$ | $6.57_{c}$ | 0.648 |
| Day 134 | $9.10_{a}$ | $5.24_{c}$ | $6.16_{b}$ | $5.46_{bc}$ | 0.265 |
| **Creatinine (mg/DL)** | | | | | |
| Day 0 | $1.369_{b}$ | $1.284_{ab}$ | $1.174_{a}$ | $1.352_{b}$ | 0.052 |
| Day 26 | $1.012_{ab}$ | $0.979_{b}$ | $0.971_{b}$ | $1.123_{a}$ | 0.041 |
| Day 99 | $1.483_{a}$ | $1.232_{b}$ | $1.281_{b}$ | $1.312_{ab}$ | 0.062 |
| Day 134 | 1.679 | 1.664 | 1.645 | 1.653 | 0.057 |

a.b.c = $p<0.05$; x.y.z = $p<0.1$; SE = Standard error

The amino acid concentration of blood serum recorded at the onset of the trial (d 1), the beginning of the grower phase (d 26), the beginning of the finisher phase (d 99) and the end of the study (d 134) is shown in Tables 14 – 17.

For most of the amino acids over all the treatments, the amino acid concentration in the beginning of the trial was at their lowest point. This is understandable seeing that the cattle were most probably in a negative energy balance, leaving their body rather in a catabolic state than an anabolic state.

Serum amino acid profiles can be affected by a number of different factors making it difficult to interpret the results. In general, an increase in blood serum concentration of any of the essential AA in response to its supplementation generally signifies that the supply of that AA exceeds the capacity for protein synthesis as dictated by the first limiting AA (Bergen, 1979). In contrast, a decrease in the serum concentration of other AA when a first-limiting AA is supplemented, indicates a better utilisation for anabolic purposes because supplementation of the limiting AA should eliminate previous limitations that the basal diet may have imposed on protein synthesis (Wessels *et al*., 1997). As lysine and/or methionine was supplemented in the RUP+L and RUP+L+M treatment. one would expect to see an increase in blood serum levels of lysine and/or methionine, but this was not the case. The supplemented treatments

had lower lysine and methionine blood serum concentrations than the con and RUP treatment, indicating that these two amino acids were limiting.

The supplementation of lysine and/or methionine did not increase growth performance (P>0.05), indicating that need for lysine and methionine wasn't fulfilled yet. These findings are supported by Papas *et al* (1984) where the lower supplemented group of 6.9 g methionine per animal per day have not shown any increases in blood plasma methionine concentrations. They only started seeing increases in blood plasma methionine concentrations when 12.9g of methionine was supplemented per animal per day. In the trial the actual amount of rumen protected lysine and methionine supplied differed as the DM intakes different than was predicted by AMTS. The actual available lysine and methionine supplied by the rumen protected amino acids is listed below. Ajipro L supplied 25% metabolizable lysine per unit of Agipro L (Texeira *et al*., 2019) and the methionine in smartamine M was 77.5% bioavailable (Blum *et al*., 1999).

- Lys Treatment
    - Starter
        - Ajipro L = 10.13g/head/day
            - Metabolizable lysine = 2.53g/head/day
    - Grower
        - Ajipro L = 12.96g/head/day
            - Metabolizable lysine = 3.24g/head/day
    - Finisher
        - Ajipro L = 13.38g/head/day
            - Metabolizable lysine = 3.34g/head/day
- Lys+met Treatment
    - Starter
        - Ajipro L = 10.64g/head/day
            - Metabolizable lysine = 2.66g/head/day
        - Smartamine M = 3.17g/head/day
            - Metabolizable methionine = 2.46g/head/day

- Grower
  - Ajipro L = 13.04g/head/day
    - Metabolizable lysine = 3.26g/head/day
  - Smartamine M = 3.87g/head/day
    - Metabolizable methionine = 3.0g/head/day
- Finisher
  - Ajipro L = 12.48g/head/day
    - Metabolizable lysine = 3.12g/head/day
  - Smartamine M = 3.81g/head/day
    - Metabolizable methionine = 2.95g/head/day

It may be that the levels of methionine and lysine supplemented is assumed to be too low to see an increase in the serum amino acid concentrations seeing that these levels were even lower than the supplemented levels by Papas *et al* (1984). Ordway *et al* (2009) also supplemented much higher levels of methionine (27-55g/d/h) than what was observed in this trail

For the RUP+L treatment where only lysine was supplemented, most of the other amino acid concentrations only decreased numerically ($P>0.05$). For the RUP+L+M treatment there was a bigger response on the other serum amino acid profiles. Isoleucine, leucine, valine, alanine, glutamic acid, proline and tyrosine was significantly ($P<0.05$) lower than the con treatment, indicating that methionine was one of the limiting amino acids in the diet. As lower levels of the other amino acids indicate that they could be used as methionine was there to complete the building blocks.

Supplementing lysine and or methionine didn't influence most of the serum amino acid concentration ($P>0.05$). The reason why we see so much numerical changes. but no statistical changes can possibly be due to a small sampling group and because the standard error was so large for most of the amino acids.

Although an optimal Lys:Met ratio of 3:1 (Chalupa & Sniffen. 2006) was fed in the RUP+L+M treatment, it was not realised in the blood serum with all the ratios being lower in the blood serum. Indicating that with the addition of methionine to the diet, more lysine was utilised seeing that the lysine was lower, and the methionine concentrations was higher in the RUP+L+M treatment compared to the RUP+L treatment.

Lysine and alanine are the most abundant amino acids in muscle protein (Bach *et al*., 2000). The RUP+L+M treatment had the lowest concentration of alanine and lysine, indicating that there may have been more muscle accretion. This was confirmed by the carcasses being much leaner.

Serum Lys concentration was only affected on d 134 where the CON treatment was higher ($P < 0.05$) when compared to RUP and RUP+L+M treatments. The concentration of serum Met was higher only on d 26 when compared to the other treatments, suggesting a slight oversupply of Met. This is supported by a numerically higher feed intake for RUP+L+M when compared to the other treatments over the first 47 days of the study. The serum concentrations of Ala, Glu, Pro and Tyr were lower on sampling d 99 for RUP+L+M when compared to treatment CON ($P < 0.05$). Similarly, on sampling d 134 the serum concentration of the EAA Ileu, Leu, Lys and Val as well as Pro and Tyr were lower for RUP+L+M compared to CON ($P< 0.05$). Texeira *et al* (2019) reported that many serum AA in steers supplemented with lysine were decreased when compared to a control diet, similar to our results. Decreased serum AA concentrations supplemented with RPLys is consistent with an increased leanness of carcass indicating that these AA have been utilised for anabolic purposes. Batista *et al* (2016) also reported a linear decrease in the plasma concentration of Leu, Ser, Tyr, and Val ($P < 0.05$) and a tendency for decreased Asn and Phe ($P = 0.07$) suggesting an increased uptake and utilisation of these AA for protein deposition.

Comparing these serum amino acid levels to the results of other studies is very difficult to do seeing that there is a lot of variation to account for. Factors contributing to the variation is as diet composition, animal type, environment, laboratory techniques, ruminal development, and microbial population development.

Table 14: The effect of supplemented Lys, Met and Urea on the amino acid profile of blood serum (Day 0)

| AA (mmol/L) | CON | RUP | RUP+L | RUP+L+M | SE |
|---|---|---|---|---|---|
| **Essential Amino acid** | | | | | |
| **Histidine** | 47.19 | 48.82 | 45.55 | 46.49 | 1.61 |
| **Isoleucine** | $81.67_{x}$ | $80.39_{x}$ | $76.56_{xy}$ | $68.69_{y}$ | 4.71 |
| **Leucine** | 107.14 | 111.71 | 99.38 | 96.28 | 6.99 |
| **Lysine** | 64.68 | 69.73 | 64.58 | 60.23 | 5.23 |
| **Methionine** | 20.23 | 21.41 | 20.65 | 19.54 | 1.13 |
| **Phenylalanine** | 52.81 | 54.79 | 50.63 | 51.43 | 2.28 |
| **Threonine** | $59.07_{ab}$ | $63.58_{ab}$ | $64.51_{a}$ | $56.16_{b}$ | 2.60 |
| **Tryptophan** | 33.67 | 39.32 | 34.47 | 37.27 | 2.56 |
| **Valine** | 198.58 | 193.06 | 187.09 | 176.00 | 10.28 |
| **Non-essential amino acids** | | | | | |
| **Alanine** | $253.84_{a}$ | $257.28_{a}$ | $215.45_{b}$ | $222.71_{b}$ | 9.23 |
| **Asparagine** | $27.04_{x}$ | $27.33_{x}$ | $25.80_{xy}$ | $23.90_{y}$ | 1.21 |
| **Aspartic Acid** | 6.61 | 6.89 | 7.38 | 7.01 | 0.61 |
| **Cystine** | 6.22 | 7.11 | 6.35 | 6.12 | 0.45 |
| **Glutamic acid** | $72.04_{ab}$ | $77.29_{a}$ | $67.57_{ab}$ | $60.19_{b}$ | 4.34 |
| **Glutamine** | $200.06_{ab}$ | $205.10_{a}$ | $194.49_{ab}$ | $186.88_{b}$ | 5.86 |
| **Glycine** | $518.72_{x}$ | $584.96_{y}$ | $546.16_{xy}$ | $550.65_{xy}$ | 22.41 |
| **Proline** | 67.90 | 70.29 | 67.80 | 66.23 | 2.28 |
| **Serine** | $84.98_{a}$ | $94.78_{b}$ | $83.89_{a}$ | $85.78_{a}$ | 2.66 |
| **Tyrosine** | $41.67_{x}$ | $40.49_{xy}$ | $41.34_{x}$ | $35.35_{y}$ | 2.37 |
| **NEAA** | $1279.09_{b}$ | $1371.53_{a}$ | $1256.22_{b}$ | $1244.83_{b}$ | 28.14 |
| **EAA** | 665.04 | 682.81 | 643.43 | 612.09 | 29.80 |
| **TAA** | $1944.13_{ab}$ | $2054.34_{a}$ | $1899.65_{b}$ | $1856.92_{b}$ | 47.61 |
| **Lys:Met** | 3.20:1 | 3.26:1 | 3.15:1 | 3.06:1 | 0.21 |

A,b,c = $p<0.05$; x,y,z = $p<0.1$; SE = Standard error; NEAA = non-essential amino acids; EAA = essential amino acids; TAA = total amino acids

Table 15: The effect of supplemented Lys. Met and Urea on the amino acid profile of blood serum (Day 26)

| AA (mmol/L) | CON | RUP | RUP+L | RUP+L+M | SE |
|---|---|---|---|---|---|
| **Essential Amino acid** | | | | | |
| **Histidine** | $66.70_{xy}$ | $64.17_{x}$ | $73.04_{y}$ | $66.31_{xy}$ | 3.02 |
| **Isoleucine** | 120.65 | 128.75 | 118.98 | 118.64 | 6.54 |
| **Leucine** | 159.84 | 163.33 | 151.92 | 149.60 | 9.49 |
| **Lysine** | 115.30 | 118.88 | 111.97 | 102.46 | 9.46 |
| **Methionine** | $33.66_{xy}$ | $31.27_{x}$ | $31.56_{x}$ | $37.09_{y}$ | 2.10 |
| **Phenylalanine** | 55.09 | 52.77 | 53.32 | 54.55 | 2.28 |
| **Threonine** | 72.72 | 85.11 | 80.78 | 78.21 | 6.99 |
| **Tryptophan** | 34.49 | 37.84 | 35.81 | 36.69 | 1.91 |
| **Valine** | 275.27 | 313.38 | 294.80 | 275.37 | 18.28 |
| **Non-essential amino acids** | | | | | |
| **Alanine** | 270.55 | 296.46 | 267.84 | 265.90 | 19.74 |
| **Asparagine** | 50.61 | 50.10 | 47.66 | 51.42 | 3.22 |
| **Aspartic Acid** | 12.08 | 11.44 | 10.46 | 10.34 | 1.50 |
| **Cystine** | $7.74_{a}$ | $8.05_{a}$ | $8.39_{a}$ | $9.17_{b}$ | 0.40 |
| **Glutamic acid** | 73.88 | 79.10 | 65.03 | 74.56 | 7.76 |
| **Glutamine** | $366.24_{x}$ | $324.46_{xy}$ | $313.49_{y}$ | $312.80_{y}$ | 19.61 |
| **Glycine** | 450.27 | 455.65 | 450.19 | 437.30 | 18.51 |
| **Proline** | 86.32 | 82.10 | 84.45 | 80.99 | 4.08 |
| **Serine** | 95.75 | 102.92 | 96.82 | 94.34 | 4.28 |
| **Tyrosine** | $73.29_{xy}$ | $63.15_{x}$ | $69.20_{xy}$ | $73.96_{y}$ | 4.32 |
| **NEAA** | 1486.73 | 1473.43 | 1413.53 | 1410.77 | 57.24 |
| **EAA** | 933.71 | 995.50 | 952.16 | 918.93 | 54.04 |
| **TAA** | 2420.44 | 2468.94 | 2365.69 | 2329.70 | 95.07 |
| **Lys:Met** | $3.45{:}1_{a}$ | $3.81{:}1_{a}$ | $3.54{:}1_{a}$ | $2.78{:}1_{b}$ | 0.18 |

a.b.c = $p<0.05$; x.y.z = $p<0.1$; SE = Standard error; NEAA = non-essential amino acids; EAA = essential amino acids; TAA = total amino acids

Table 12: The effect of supplemented Lys. Met and Urea on the amino acid profile of blood serum (Day 99)

| AA (mmol/L) | CON | RUP | RUP+L | RUP+L+M | SE |
|---|---|---|---|---|---|
| **Essential Amino acid** | | | | | |
| **Histidine** | $74.51_{x}$ | $75.41_{x}$ | $84.41_{y}$ | $79.31_{xy}$ | 3.61 |
| **Isoleucine** | 110.28 | 108.69 | 99.98 | 101.72 | 5.58 |
| **Leucine** | 164.32 | 161.29 | 155.79 | 156.52 | 7.37 |
| **Lysine** | 106.19 | 101.47 | 98.22 | 90.19 | 7.42 |
| **Methionine** | 35.85 | 36.58 | 35.10 | 36.63 | 2.20 |
| **Phenylalanine** | 53.43 | 55.76 | 52.64 | 53.28 | 1.89 |
| **Threonine** | 64.84 | 73.27 | 68.86 | 71.99 | 5.81 |
| **Tryptophan** | $37.73_{xy}$ | $42.24_{x}$ | $36.44_{y}$ | $41.41_{xy}$ | 2.10 |
| **Valine** | 265.77 | 263.02 | 253.54 | 262.12 | 12.84 |
| **Non-essential amino acids** | | | | | |
| **Alanine** | $255.69_{a}$ | $258.62_{a}$ | $240.49_{ab}$ | $230.85_{b}$ | 8.09 |
| **Asparagine** | 44.37 | 44.50 | 45.21 | 44.25 | 3.17 |
| **Aspartic Acid** | 7.72 | 6.36 | 5.88 | 5.53 | 1.05 |
| **Cystine** | 7.72 | 7.44 | 9.11 | 8.46 | 0.74 |
| **Glutamic acid** | $32.60_{x}$ | $30.62_{xy}$ | $29.54_{xy}$ | $25.05_{y}$ | 2.89 |
| **Glutamine** | 485.51 | 483.41 | 486.43 | 468.04 | 23.37 |
| **Glycine** | 341.11 | 332.47 | 352.16 | 336.02 | 21.03 |
| **Proline** | $81.73_{x}$ | $78.44_{xy}$ | $80.59_{xy}$ | $75.24_{y}$ | 2.43 |
| **Serine** | 87.89 | 87.80 | 86.28 | 85.07 | 4.21 |
| **Tyrosine** | 69.97 | 63.38 | 65.69 | $64.75_{b}$ | 4.06 |
| **NEAA** | 1414.31 | 1393.04 | 1401.39 | 1343.24 | 50.12 |
| **EAA** | 912.91 | 917.73 | 884.98 | 893.19 | 42.03 |
| **TAA** | 2327.23 | 2310.77 | 2286.37 | 2236.43 | 78.29 |
| **Lys:Met** | $2.96{:}1_{x}$ | $2.81{:}1_{xy}$ | $2.78{:}1_{xy}$ | $2.47{:}1_{y}$ | 0.17 |

a.b.c = $p<0.05$; x.y.z = $p<0.1$; SE = Standard error; NEAA = non-essential amino acids; EAA = essential amino acids; TAA = total amino acids

Table 13: The effect of supplemented Lys. Met and Urea on the amino acid profile of blood serum (Day 134)

| AA (mmol/L) | CON | RUP | RUP+L | RUP+L+M | SE |
|---|---|---|---|---|---|
| **Essential Amino acid** | | | | | |
| **Histidine** | 73.42 | 67.10 | 62.63 | 65.46 | 6.35 |
| **Isoleucine** | $109.95_{a}$ | $98.00_{a}$ | $99.45_{a}$ | $90.11_{b}$ | 4.37 |
| **Leucine** | $178.48_{a}$ | $158.41_{a}$ | $161.76_{a}$ | $146.31_{b}$ | 9.76 |
| **Lysine** | $111.88_{a}$ | $96.03_{b}$ | $101.65_{ab}$ | $94.36_{b}$ | 4.86 |
| **Methionine** | 39.21 | 36.5 | 36.64 | 37.78 | 2.02 |
| **Phenylalanine** | 57.49 | 59.71 | 57.91 | 57.09 | 3.08 |
| **Threonine** | 76.76 | 72.37 | 74.23 | 75.92 | 5.85 |
| **Tryptophan** | $47.84_{xy}$ | $49.55_{x}$ | $45.17_{y}$ | $44.63_{y}$ | 1.69 |
| **Valine** | $268.45_{a}$ | $235.81_{ab}$ | $238.48_{ab}$ | $217.05_{b}$ | 12.49 |
| **Non-essential amino acids** | | | | | |
| **Alanine** | $236.47_{ab}$ | $247.82_{a}$ | $225.48_{ab}$ | $209.99_{b}$ | 11.83 |
| **Asparagine** | 51.45 | 47.18 | 49.13 | 45.60 | 2.51 |
| **Aspartic Acid** | 8.78 | 8.67 | 8.12 | 8.45 | 0.66 |
| **Cystine** | 9.03 | 9.36 | 9.90 | 9.10 | 0.63 |
| **Glutamic acid** | $91.55_{ab}$ | $102.97_{ab}$ | $104.38_{a}$ | $81.50_{b}$ | 7.58 |
| **Glutamine** | $316.08_{x}$ | $306.60_{xy}$ | $298.41_{xy}$ | $269.21_{y}$ | 17.17 |
| **Glycine** | $332.88_{a}$ | $386.29_{b}$ | $350.32_{ab}$ | $376.75_{ab}$ | 15.99 |
| **Proline** | $94.67_{a}$ | $87.53_{ab}$ | $90.01_{ab}$ | $83.36_{b}$ | 3.42 |
| **Serine** | 89.39 | 91.58 | 89.64 | 93.10 | 2.46 |
| **Tyrosine** | $76.13_{a}$ | $66.20_{ab}$ | $72.70_{ab}$ | $64.15_{b}$ | 3.88 |
| **NEAA** | $1306.44_{xy}$ | $1354.21_{x}$ | $1298.08_{xy}$ | $1241.20_{y}$ | 41.70 |
| **EAA** | $963.47_{a}$ | $873.48_{ab}$ | $877.90_{ab}$ | $828.71_{b}$ | 41.95 |
| **TAA** | $2269.91_{x}$ | $2227.69_{xy}$ | $2175.98_{xy}$ | $2069.91_{y}$ | 72.87 |
| **Lys:Met** | 2.85:1 | 2.66:1 | 2.77:1 | 2.5:1 | 0.14 |

a.b.c = $p<0.05$; x.y.z = $p<0.1$; SE = Standard error; NEAA = non-essential amino acids; EAA = essential amino acids; TAA = total amino acids

## 5.3. Carcass parameters

The effect of supplemental RUP and RPAA on carcass parameters are shown in Table 18. The subcutaneous fat thickness measured over the13$^{th}$ rib was significantly lower ($P < 0.05$) in carcasses from the RUP+L+M treatment compared to the other three treatments ($P< 0.05$). However, subcutaneous fat thickness did not differ between the CON, RUP or RUP+L treatments. In a study conducted by Heiderscheit & Hansen (2020) they also found the backfat thickness varied between 13-15mm, which is in line with our study. Accordingly, the channel fat mass of carcasses from the RUP+L+M treatment was significantly lower than those from the CON ($P=0.008$), and RUP+L ($P= 0.04$) treatments. It is clear that carcasses from RUP+L+M treatment contained less fat than the other treatments. In a study conducted by Cantalapiedra-Hijar *et al*., (2020) young bulls fed 13 % CP diets with methionine being 2.0% of MP showed a reduction in the total internal fat. This is inline of what we saw in this study.. Burris *et al* (1976) reported a linear increase in in the N retention of feedlot steers that were abomasally infused with lysine. In our study Lys and Met together resulted in a leaner carcass suggesting that Lys and Met supplementation as well as the ratio may be important factors to consider if the consumer demands leaner carcasses. This may be beneficial for feedlots striving to provide better quality carcasses to abattoirs. The accumulation of channel fat in carcasses is a wasteful aspect of intensive cattle feeding, so lower channel fat may improve the efficiency of feedlot production.

The dressing percentages must be interpreted with caution as it was calculated based on the on the last live weight of the animals 2 days before slaughtering. The animals would have lost at least 15-20 kg due to rumen emptying. Dressing percentage was higher in the RUP+ L treatment ($P<0.05$) compared to the RUP treatment but did not differ from the CON and RUP+L+M treatments. The practical significance of these small differences is probably negligible.

Feeding a ration higher in urea may increase the concentrations of ammonia transported to the liver, resulting in the metabolism of more urea and excretion of the excess ammonia via urine. Our results support this hypothesis as the weights of the livers from cattle on the CON treatment tended to be heavier than the RUP ($P=0.09$), RUP+L ($P=0.06$) and RUP+L+M ($P=0.07$) treatments Carcass compactness tended to be higher in the CON treatment compared to the RUP ($P=0.06$) and RUP+L+M ($P=0.07$) treatments which indicated more muscling per cm of carcass length. These differences were small and probably of little practical significance.

As mentioned in the introduction, to compare South African feedlot studies was studies from the USA is a difficult matter as they work with heavier animals entering and leaving the

feedlot. So, to compare carcass characteristics of feedlot cattle where rumen protected amino acids is difficult as there isn't a lot of South African research done on this matter, but hopefully this data can be used in following studies to come

Table 14: The effect of supplemented Lysine. Methionine and Urea on carcass parameters

| Parameter | CON | RUP | RUP+L | RUP+L+M |
|---|---|---|---|---|
| Warm carcass mass (WCM) | $299.73_{y}$ | $289.51_{x}$ | $294.87_{xy}$ | $291.39_{xy}$ |
| Cold carcass mass (CCM) | $295.12_{a}$ | $283.75_{b}$ | $288.56_{ab}$ | $285.91_{ab}$ |
| Fat Score | $2.43_{x}$ | $2.30_{xy}$ | $2.27_{xy}$ | $2.18_{y}$ |
| Dressing % | $57.73_{ab}$ | $56.85_{a}$ | $58.26_{b}$ | $57.83_{ab}$ |
| Conformation | $3.36_{ac}$ | $3.63_{ab}$ | $3.70_{b}$ | $3.26_{c}$ |
| $13^{th}$ rib fat thickness | $14.56_{a}$ | $14.57_{a}$ | $14.87_{a}$ | $12.07_{b}$ |
| Channel fat surface ($mm^3$) | $13717_{a}$ | $12631_{ab}$ | $13212_{a}$ | $11576_{b}$ |
| Channel fat mass (g) | $12619_{a}$ | $11620_{ab}$ | $12155_{a}$ | $10650_{b}$ |
| Liver mass | $6.179_{x}$ | $5.850_{y}$ | $5.803_{y}$ | $5.812_{y}$ |
| Carcass length | $124.19_{a}$ | $123_{ab}$ | $122.03_{b}$ | $124.21_{a}$ |
| Compactness | $2.376_{x}$ | $2.307_{y}$ | $2.363_{xy}$ | $2.301_{y}$ |
| Liver/CCM | 0.021 | 0.021 | 0.020 | 0.020 |

A,b,c = $p<0.05$; x,y,z = $p<0.1$;

## 5.4. Rumen scoring

The rumen grading is presented in Table 15. The CON treatment had the best rumen scores. suggesting that they were better able to ferment their feed and to absorb the volatile fatty acids. The RUP treatment had severe rumen lesions as depicted in figure 6. The RUP+L+M treatment has the worst overall rumen scoring with 45% having moderate rumen damage.

The reason for the RUP+L+M treatment having poorer rumen scores and lower feed intake may had a negative effect on the trial outcome.

Table 15: Rumen health of the 4 treatments

| Grading | Unit | CON | RUP | RUP+L | RUP+L+M |
|---|---|---|---|---|---|
| Normal | % | 70 | 60 | 65 | 50 |
| Mild | % | 10 | 10 | 5 | 5 |
| Moderate | % | 20 | 20 | 30 | 45 |
| Severe | % | 0 | 10 | 0 | 0 |

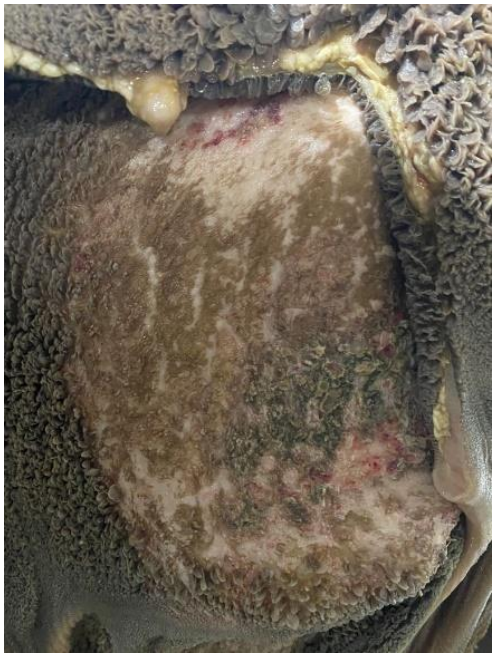


Figure 5: Rumen that had severe damage

## 5.5. Morbidities and mortalities

The trial had 1 mortality and 4 morbidities. The mortality was in the Urea treatment. the animal was pulled and treated for bloat like conditions. The following day the animal died. and the post-mortem determined that the animal had a perforating abomasal ulcer. Two of the morbidities was identified early in the trial and it was determined that they had Bovine Viral Diarrhoea (BVD). The fourth morbidity was a chronic limb impairing the animal's performance. it was determined that the animal had an abscess in his left buttocks. The last morbidity was due to pneumonia to close to slaughter to treat as there wasn't sufficient time for withdrawal of mediation. hence animal was sent for slaughter.

There were other animals during the trial that was limping. had injured tails. or had injuries due to riding each other. These animals were treated and had no severe impact on their growth performance hence they were kept in the trial.

# Chapter 5

# Financial Implications

Table 16: Profitability of the four different treatments

| **Calf weight** | **Con** | **RUP** | **RUP+L** | **RUP+L+M** |
|---|---|---|---|---|
| Calf weight | 251.1 kg | 250.6 kg | 252.4 kg | 250.7 kg |
| Purchase price (16/10/2020) | R 36.93 | R 36.93 | R 36.93 | R 36.93 |
| **Average price per calf** | **R 9 273.12** | **R 9 254.65** | **R 9 321.13** | **R 9 258.35** |
| R/ton starter feed | R 2 322.89 | R 2 724.95 | R 2 697.44 | R2 775.72 |
| Total starter feed price | R 666.65 | R 769.74 | R 741.81 | R 797.13 |
| R/ton grower feed | R 2 601.81 | R 2 847.50 | R 3 047.53 | R 3 116.23 |
| Total grower feed price | R 2 427.00 | R 2472.24 | R 2 762.70 | R 2 843.03 |
| R/ton finisher feed | R 2 919.38 | R 3 020.32 | R 3 328.09 | R 3 405.31 |
| Total finisher feed price | R 1 364.17 | R 1 321.39 | R 1 438.10 | R 1 392.84 |
| **Total feed price** | **R 4 457.82** | **R 4 563.37** | **R 4 942.61** | **R 5 032.99** |
| Carcass weight | 295.12 kg | 283.75 kg | 288.56 kg | 285.91 kg |
| Carcass sell price (1/03/2021) | R 51.65 | R 51.65 | R 51.65 | R 51.65 |
| **Carcass price** | **R 15 242.95** | **R 14 655.69** | **R 14 904.12** | **R 14 767.25** |
| **Nett income per calf** | **R 1512.01** | **R 837.67** | **R 640.38** | **R 475.91** |

Table 16 depicts the profitability of the four treatments. The calf purchase price was approximately the same since there was little difference in starting weights. The RUP+L+M treatment resulted in a lower feed intake compared to the other treatments (RUP=9.24 kg//d, CON=9.59 kg//d, RUP+L=9.14 kg//d, RUP+L+M=9.07 kg/d). The RUP+L+M treatment was the most expensive per ton of feed due to the high cost of RPAA . The lower feed intake of the RUP+L+M treatment, however, was not sufficient to offset the higher cost per ton.

The combination of lowest feed cost and highest carcass mass resulted in the CON treatment being the most profitable. The margin over feed cost was the lowest for the RUP+L+M and RUP+L treatments (R 475 and R 640 respectively and the highest for the CON and RUP treatments (R 1512 and R 837 respectively)

# Chapter 6

# Conclusion

Overall supplementation with additional RUP or RPAA did not improve ADG or FCR suggesting that a diet formulated according to NRC standards with sufficient energy, RDP and effective fibre that promotes optimal rumen fermentation meets the MP requirements for a mean ADG of 1.89 kg/d and FCR of 4.95 kg feed/kg gain. Results furthermore suggest more efficient utilisation of N and AA utilisation with the supplementation of RPAA resulting in leaner carcasses. Further research into the effect of RPAA supplementation in younger steers weighing less than 200kg may have merit as well as a trial where RPAA is only supplemented in the starter vs the grower vs the finisher period.

# Chapter 8

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