Mineral and Nutrient Evaluation of Horse Feeds and Fodder in South Africa

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BSc Agric (University of KwaZulu-Natal)

Submitted in partial fulfilment of the requirements for the degree of

Master of Science in Agriculture

In the

Discipline of Animal and Poultry Science
School of Agricultural Sciences and Agribusiness
College of Agriculture, Engineering and Sciences
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Pietermaritzburg Campus

December 2015

Abstract

Little information is available on the nutrient, and particularly the mineral provision of fodder and feed in the South African horse industry. Rational feeding models are not applied, and inadequacies in the ration are compensated for by increments in concentrate feed intake. This constitutes a violation of nutritional physiology in the hindgut fermenter, and consequences of high concentrate feeds are discussed. Nutrient provision from the fodder portion of the ration is not considered when feed formulation and raw material selection occur. Consequently, feed and fodder samples were collected from a variety of sources and subjected to mineral and nutrient analyses to elucidate the extent of the problem as it pertains to the meeting of requirements in horses of different life stages and work rates. 99 fodder samples were analysed and Principle Component Analyses (PCA) were conducted. Three statistically diverse groups of fodder were identified - kikuyu and lucerne contain high digestible energy and crude protein content which differentiates them from other grasses. Mineral provision from the three groups is diverse. There is justification for developing fodder specific feeds for horses based on these fodder types.

64 concentrate horse feeds were analysed by PCA to determine groups of similar nutrient content. Balancer and fibre feeds could be differentiated from other feeds based on their fibre and CP content. The balance of the feeds could not be segregated into their life stage or work purpose groups and statistically fell into the same feed group. Correlation matrices between advertised and actual nutrient content revealed inconsistencies between feed factories. Mineral provision in the feeds is a function of the factory they are produced in and not as a function of the life stage and work purpose of the feeds. Major mineral provision in horse feed is erratic and 15 out the 64 feeds exhibited inverse Ca:P ratios and very few have sufficient Mg to balance the Ca:Mg ratio required by horses. Horse feeds contain excess Fe and are often deficient in Cu and Zn while Mn provision is adequate. It is evident that brand or factory plays a more important role in mineral nutrition of horses, than life stage or work

rate. Feed formulation strategies appear to be least cost based with little regard for horse requirements evident in the final feed products.

Ration evaluation revealed that grasses are able to provide sufficient DE and CP for horses in light work while kikuyu and lucerne can support horses up to the moderate work category. All fodder types require mineral supplementation to provide absolute mineral levels and balance between minerals to horses.

A comparison of five equine life stage reconstituted rations were then constructed and work ration scenarios demonstrated that DE and CP provision in most instances was sufficient, although CP overprovision did occur. Mineral levels were erratic and in the stud and race groups, feeds produced inverse Ca:P ratios. This could affect skeletal integrity and cause lameness – two issues often found in racehorses, leading to wasted training days and horse breakdowns. Major overprovision of Fe occurs in all the rations with concomitant deficiencies of Cu and Zn. This imbalance could have negative consequences in the horse in terms of immune and anti-oxidant systems.

It is evident from the study that feeds are poorly formulated and feeding strategies currently employed in SA are flawed in their provision of health-promoting nutrition to horses. New strategies and feed formulation goals need to be investigated and the feeding of horses in SA needs to be restructured to respect the horse as a hindgut fermenter with an absolute requirement for fodder and to provide correct mineral balance in rations.

Candidate's Declaration

I, Sheldene Kerry Horne, declare that

The research reported in this thesis, except where otherwise indicated, is my original research. This thesis has not been submitted for any degrees or examination at any other university. This thesis does not contain other persons' data, pictures or graphs or other information unless specifically acknowledged as being sourced from other persons.

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Supervisor’s Declaration

I hereby release this dissertation for examination in my capacity as supervisor.

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December 2015

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Acknowledgements

I would like to extend my sincere appreciation to the following people without whom this study would have been impossible:

Jodi, Nick and Albert you started me on the path of enquiry, leading me to seek answers as to why our horses are floundering in the modern environment. Without your influence, I would never have opened my mind up to the possibilities waiting for me.

Sherene Naicker at Cedara Feed Laboratory for your willingness to sort out special requests, often on short notice, and answering questions and queries during the years of sample submissions. You and your team provide a dedicated and efficient service which is hard to find nowadays.

Marion, my friend and supervisor – for the financial assistance and for believing in me and picking me up when the going got tough. You can teach an old dog new tricks – I hope I have learnt to be succinct and discursive. Marion - you are an inspiration!!

Debbie and Cat for your support, advice and friendship in the last two years.

Phillemon for your willingness to assist wherever you could.

Fortune and Bernie from the Mastering Masters Programme – the courses and training days you offer, have made researching and writing this thesis so much easier.

To my family – Mom and Dad, Kelvin and Cath, without your support at home I would not have been able to follow my dreams.

To my daughters Hayley and Taylor for coping so well with a distracted and often absent mother.

Trish, Ryan and Jess for opening up your home for me and providing my home away from home.

Then to Graeme – your unfailing love and support mean the world to me! Without you, this thesis would merely be a pipe dream instead of my reality.

List of Abbreviations

ADF	Acid Detergent Fibre
AOAC	Association of Official Analytical Chemists
BW	Body Weight
CP	Crude Protein
DE	Digestible Energy
DM	Dry Matter
DMI	Dry Matter Intake
EMS	Equine Metabolic Syndrome
KER	Kentucky Equine Research Unit
MJ	Mega Joules
NDF	Neutral Detergent Fibre
NFE	Nitrogen Free Extract
PCA	Principle Components Analysis (Genstat v14 2014)
SA	South Africa
SI	Small Intestine

Table of Contents

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Figures

Chapter 2

Chapter 3

CHAPTER 4

Chapter 5

Overview

Mineral status in horses could be measured in the blood for some minerals, and in the liver through biopsies. However, feed evaluations are becoming increasingly popular for the purpose of determining mineral and other nutrient ingestion. Because horses are kept for recreational, companion and competitive purposes, the meeting of requirements and optimal equine nutrition for predictable outcomes, is now a focus of equine feeding strategies. But rations fall short, and horses do not perform as expected.

In the SA feed industry, however, the evaluation of the integrity of the feed formulation has become a retrospective exercise. In conventional animal intake modelling theory, intakes of a nutrient are driven by the requirement for it (Emmans and Kyriazakis, 2001). In the context of the horse, increased intakes of high nutrient density concentrate feeds are provided, in an attempt to make sure that nutrients are provided. This is evidenced by the array of nutritional supplements and feeds on the market in this country and abroad. A horse feed does not seem to do what it should.

This dissertation focuses on aspects of mineral nutrition in SA concentrate horse feeds and fodder types. By identifying correct nutrient requirements, the nutrient intakes are assessed in relation to what the horse actually needs. Intake then is modelled on the satisfaction of the first limiting nutrient, as opposed to the excess provision of feed in order that nutrient requirements might be met.

The objectives of the thesis are:

1. To review the body of scholarship pertaining to the provision of feeds and forages to horses,
2. To assess the fodder types available to horses in South Africa, and determine general nutrient contents, and in particular, mineral contents of types of fodder,
3. To determine the nutrient and specifically the mineral content of concentrate horse feeds,
4. To assess the concurrent provision of feeds and forages in respect of mineral provision to horses, and relate this to the documented requirements of the horse.

To this end, this dissertation will attempt to discern the feed formulation strategy that factories seem to obfuscate in the provision of excess feed. Principal Component Analysis is a multivariate technique which reduces variation by finding a linear combination of a set of classifying variables that reduce the dimensionality of the data. It can successfully identify both classifying variables and construct concomitant groupings of data. These will elucidate average nutrient contents and variables of importance in several horse concentrate feeds and fodder types. Hence, typical rations can be evaluated for mineral and nutrient provision.

The South African (SA) horse population is estimated to be 300 000 with approximately 60 000 registered purebred horses of which 20 000 are Thoroughbred racehorses (KER, 2011; European Commision, 2013). Horse feeds sold from AFMA registered companies currently total 38000t per annum with non-AFMA registered companies adding unknown tonnages to this. The feed market primarily supplies stabled sport and racehorses who are fed large amounts of concentrate per day, with hay as their sole fodder source.

Horses are kept under a variety of management systems in SA. Farm and rural horses graze to fulfil 100% of their daily needs and leisure horses eat a minimal amount of concentrate with pasture or hay making up the bulk of the ration. Other horses are able to graze during the day and are stabled and fed hay at night, while a large number of high value sport and pleasure horses are kept in urban areas where there is limited turnout space. These horses are stabled for large parts of the day and rarely have access to grazing. They are generally fed a high proportion of concentrate feed in their ration with limited hay provision. Thoroughbred racehorses are stabled throughout the day and night and are fed 80% of their ration in concentrate feed with small amounts of hay offered daily (Hackland, 2007).

For the purposes of this dissertation, the following terms are used:

Ration – the total daily intake of a horse consisting of fodder and feed

Feed – concentrate (bucket) feed manufactured for horses and fed in varying amounts in two to three daily meals to horses

Fodder – Provided as

1) Grazing from indigenous veld or pasture species which horses are able to forage and selectively graze, generally consisting of limited species of grass or improved pasture species,

2) Hay sun dried and baled fodder mainly from single grass species or lucerne. Indigenous veld grasses may have a limited species mix of indigenous grasses depending on the state of the veld from which it is cut.

Provision of the daily ration of feed and fodder to the hindgut fermenter, is not entirely about the horse itself. There is an entire discourse on consequence of overprovision and environmental impact which the horse fraternity has yet to engage on. The consequence to the horse itself should be a primary objective in the development of any rational feeding model. This dissertation will highlight mineral nutrition in the horse, and feeding strategies that will ameliorate the consequence of an absent feeding model in horses.

Chapter 1. Introduction

"The only aim must be the horse's welfare. Our challenge is to develop species-specific, health-promoting nutrition to ensure a long, well-fed life." Meyer (1999)

With the rapid increase of disease in humans and animals in the last century, optimal mineral nutrition could play a fundamental role in disease prevention. Worldwide, concern is growing over falling nutrient content in soils, foods and feeds due to nutrient movement from agricultural to urban areas resulting in poor nutrient recycling (Jones *et al.*, 2013) and decreasing soil nutrient content (Miller and Welch, 2013). As global pressure increases to produce large quantities of food to support rising population levels, the nutrient density of agricultural products has been ignored in favour of increased yields, resulting in increases in malnutrition (Jones *et al.*, 2013; Miller and Welch, 2013).

Due to the increased reliance on many cereal grains for use in horse feeds and by-products such as brans, oil cakes and beet and fodder which are grown under declining nutrient conditions, horses may be experiencing similar nutritional deficiencies as humans are. This is further compounded by the lack of variety in most modern horse diets. The "urbanisation" of many horse populations has created markets for hay and feed producers who send their product off-farm, driving this nutrient exportation to urban or semi-urban areas, with no nutrient returns to the farm. The rising costs of fertiliser and fuel have reduced the amount of fertiliser and lime applications used in hay and crop production potentially decreasing the nutritional value of these products further (Farina, 2014).

With inherent difficulties in the accurate determination of mineral content in horses via blood samples (NRC, 2007) and high cost and risks involved in liver biopsies, feed analyses and ration evaluation is becoming more attractive in determining accurate feed and nutrient intakes

(NRC, 2007; Kellon, 2008a; Becvarova *et al.*, 2009). Feed analyses are affordable, fast and easily interpreted. When coupled with the National Research Academy (NRC) guidelines for nutrient requirements in horses, they can be used to formulate equine diets to reduce production losses, increase performance and to optimise the use of nutrients in production systems. This obviously depends on the construction of a matrix of relevant nutrient values that are situationally and geographically specific.

Kentucky Equine Research unit (KER), recommends a minimum fodder intake on a dry matter (DM) basis of 1% to 1.5% of body weight (5 - 7.5 kg for a 500 kg horse), while other recommendations are for a minimum of half the caloric intake to come from fodder (Kohnke, 1998) to ensure equine gut health. Feed companies recommend feeding levels of concentrate far in excess of this with feeding recommendations often given at 4 – 10 kg which leaves little room for correct fodder provision in the diet. With the potential for declining nutrient content in the raw ingredients and fodder, are bagged feeds actually providing optimal, balanced nutrition in horses and are feeds formulated to suit the various fodder types used in the SA horse industry?

With the need for better feed efficiency and less environmental impact from all animal management systems (Bott *et al.*, 2013; Gordon *et al.*, 2013) the horse industry in SA needs to ensure the provision of optimum nutrition for all horses in all life-stages to provide them with the greatest opportunity to perform at their peak and to eliminate wastage of nutrients which may contaminate the environment. As many minerals are known environmental pollutants (NRC, 2007; Parvage *et al.*, 2015) their inclusion in the diets of horses must be optimal for the horse yet low enough to prevent contamination of stable waste products with high levels of these minerals. It is often neglected in the SA context that consequences to excess provision of protein and minerals can have toxic and cause devastating long-term consequences for the environment (Parvage *et al.*, 2015; Westendorf and Williams, 2015).

As essential minerals are not produced internally, horses, which are hindgut fermenters, rely on their diet for all the minerals they require. In SA, many horses are kept in urban areas with very little grazing and are fed the same feeds and fodder throughout much of their lives.

Horses in more rural areas have access to grazing but are seldom offered much choice in fodder types nor do they have free access to choose different plants or grass species to graze due to veld degradation or monoculture pastures. With far longer lifespans than production animals, any excess or deficiency in the diet will, over time become a serious nutritional disorder and may affect the health and production potential of the horse (Kellon, 2008a).

It is the intention of the current study to contextualise the intake of horse feeds and fodder types, in respect of their mineral contents. Current production systems and fodder feeding and production practices for horses need to be re-evaluated to establish norms and requirement strategies need to be identified to improve mineral nutrition in the horse.

The entire context of horse keeping has changed. Harris (1998) explains how the horse as a traction and war transport animal has evolved to one for sport and recreation. In the SA context, the expense of keeping a recreational animal limits who can own one, but also, does not preclude the use of the horse still for transport and traction. The type of work a horse does has a different definition in a rural and an urban context. When describing the horses in this study, it is the recreational and sport horses and their work levels that are referred to. The provision of concentrate feed to working horses is best qualified in terms of finances and availability of feeds to the owner. Approximately fifteen factories attempt to provide best nutrition to sport and recreation horses. What these factories are cognisant of in terms of feeding strategies is the purpose of this study.

Chapter 2: Review of the literature

The welfare of the recreational horse is a function of the management practices the horse undergoes (Hemsworth *et al.*, 2015). Feeding is a major role player in horse welfare and needs to be correctly undertaken to ensure optimal supply and maximum use of nutrients with as little environmental impact as possible. Excess nutrients such as nitrogen, P and trace minerals in the diet are excreted from the horse and can have a negative impact on the environment. It is therefore essential to balance nutrient provision to the horse with effective management of horse waste products (Bott *et al.*, 2013; Parvage *et al.*, 2015; Westendorf and Williams, 2015). In many other species the overprovision of nutrients and a C:N imbalance leads to the excess elimination of the excess nutrients leading to eutrophication (Young, 2011; Westendorf and Williams, 2015)

An understanding of the digestive processes and nutritional needs of the horse, is fundamental in efficient feed provision. Horses are classified as monogastric, hindgut fermenters which allows them to utilise higher levels of fibrous feedstuffs more efficiently than other monogastric species. Their digestive systems are designed for regular intakes of small amounts of high-fibre, low-quality fodder throughout the day (Pagan, 2000; Kellon, 2008b; Frape, 2010). Modern feeding practices largely ignore these principles with the provision of two or three large concentrate based meals fed daily with less emphasis on fodder inclusions in the diet (Thorne *et al.*, 2005). Many stereotypical behaviours can result from incorrect feeding practices which negatively impact the horse's health and wellbeing such as cribbing, weaving and wood chewing (Pagan, 2000; Freire *et al.*, 2009; Ellis *et al.*, 2015) while physical issues like colic, gastric ulceration, hindgut acidosis and laminitis occur more frequently with lower fibre diets (Harris *et al.*, 2005; Longland, 2012; Glunk *et al.*, 2013). The more natural a horse's feeding behaviour is, with choice of *ad libitum* fodder, small more frequent meals and the continual ability to ingest feed or fodder, the higher the horses nutrient intake will be and improvements in the wellbeing of the horse will occur (Julliand *et al.*, 2008).

Concentrate feeds for horses often provide excesses of certain nutrients while being deficient in others (Horne and Young, 2014). Despite marketing strategies trying to convince horse owners that the feeds supply everything a horse needs in a balanced manner. Fodder should make up the bulk of a horse's diet with additions of small amounts of concentrate to supplement what is missing from the feed (Pagan, 2000; Frape, 2010). Instead the majority of stabled horses are often subjected to large concentrate meals fed once or twice a day with minimal fodder intake (Pagan, 2000). Feeding goals for life stages of horses need to be defined and used in raw material selection to ensure total dietary intakes of horses are suited to their needs (NRC, 2007).

2.1. Fodder in horse rations

Fodder should form the basis of any horse ration with grain and supplementary feeds being provided only to fill the horse's requirements which are not met by the fodder (Pagan, 2000; Coenen *et al.*, 2011). As hindgut fermenters, horses have a requirement for roughage in the diet provided by fodder and should this requirement not be met, the horse is prone to physical and behavioural issues (Ellis *et al.*, 2010; Coenen *et al.*, 2011). In SA, fodder provided to horses varies as per the geographical location of the horse and the management practices under which they are kept. In the urban areas, large populations of sport and recreational horses are stabled with limited turnout and grazing opportunities. The sole fodder source for these horses is hay with a large proportion of their ration consisting of concentrate feeds - a practice which is common worldwide in intensively managed sport horses (Freire *et al.*, 2009; Verhaar, 2014). In rural areas horses have access to grazing and in certain cases this provides their only feed source while others graze during the day, are stabled at night and fed hay with two meals of concentrate.

Various fodder types have unique characteristics defining their nutritive quality due to environmental and management practices which occur during their growth. These include soil mineral content and availability, growth stage at grazing / harvest, temperature, rainfall and season (Longland, 2012; Virkajarvi *et al.*, 2012). The quality of a fodder can either be defined as the function of its ability to fulfil the needs of the horse eating it (Longland, 2012) or as a function of its nutritive value including digestible energy (DE) and protein content, its hygienic quality and lack of toxic materials (Virkajarvi *et al.*, 2012).

Pasture and grazing management can affect the quality of the fodder and should be optimised to prevent overgrazing and a drop in nutritional quality (Siciliano, 2015). Young, actively growing pastures or grasses contain different CP, DE, fibre and mineral content to mature grasses (Kellon, 2008a; Staniar *et al.*, 2010; Virkajarvi *et al.*, 2012) and the stage of growth of a grass will determine the mineral supplementation needed for horses eating it. As a grass matures it becomes more fibrous, less palatable and less digestible (Siciliano, 2015). For example, the crude protein (CP) quality is related to the amount of various amino-acids absorbed from the fodder in the small intestine and declines with maturity and fibre content of grasses (Virkajarvi *et al.*, 2012).

Fodder generally has low P content (0.23 - 0.33%) and high potassium content (1 - 3%). Leguminous fodder of equal maturity contains high protein (15 - 20% CP) and Ca content (1.2 - 1.8%) with higher DE than grasses which generally contains between 0.3 - 0.7% Ca, and 5 - 20% CP (Siciliano, 2015). Cu and Zn content in fodder tends to be low while Mn content can vary widely. Fe content is typically high and Siciliano (2015) reports that it is of little consequence to horses as it is poorly absorbed. A search of the literature shows little horse related research has been carried out in terms of trace mineral availability from fodder and Kellon (2008a) suggests that the Fe in the fodder is well absorbed by horses and can have negative health consequences, especially in the absence of sufficient Zn and Cu to counter this overprovision. This theory was supported in investigations into liver trace mineral content of horses in KwaZulu-Natal where 70% of studied horses had excess Fe and 65% were deficient in Zn across a range of Fodder : Concentrate diets (Horne, 2015). NRC (2007) state that “the ratios of all minerals should be taken into consideration, as minerals often influence the absorption, metabolism, and/or excretion of other nutrients”. Due to the high Fe content encountered in many fodder types it would therefore be prudent to reduce the overprovision of Fe or increase the provision of antagonistic minerals to counter this excess dietary supply.

As it stands the most recent comprehensive SA fodder norms for use in the feed industry were compiled by Bredon *et al.* (1987), Meissner and Paulsmeier (1995) and Meissner (1997) who reported macro-nutrient content for a range of SA fodder types (Table 2.1). These norms contain very limited information in terms of mineral content and Bredon *et al.* (1987) do not give ADF and NDF figures. Without current information regarding the nutrient content of the various fodder types available to horses in SA, it is impossible to formulate complementary feeds to provide a balanced ration for horses.

2.1.1. South African hay species

The dominant preserved fodder types in SA are sun dried hays with very little haylage or artificially dried hay being produced. Fodder species used for hay production for horses tend to be predominantly *Eragrostis curvula* (Weeping love grass or eragrostis), *Eragrostis tef* (teff), *Medicago sativa* (lucerne), *Avena sativa* (oat-hay), (Van Dyk and Neser, 2000) and a variety of indigenous veld grasses (called Red grass or veld hay) are fed. Less common varieties such as *Chloris gayana* (Rhodes grass), *Digiteria eriantha* (Smutsfinger grass) and *Cynodon dactylon* (Bermuda grass) are grown and fed in certain areas.

Eragrostis curvula (Weeping love grass or eragrostis) is a tufted, perennial, native grass of southern Africa used mainly for hay or pasture production in the Drakensberg and Highveld areas (Meissner, 1997). Yields vary from an average of 3 - 10t/Ha in dry areas with low fertiliser applications to 20 - 30t/ha when it is well watered and fertilised (FAO *et al.*, 2015). In the eastern areas of SA where it is grown, it is a popular hay for horses (Iwanowski, 1987; Van Dyk and Neser, 2000). Palatability drops with age as fibre content increases and older cuttings will be less acceptable than younger cuttings. Protein content also declines with age and varies from 5.6 - 18% depending on cutting time and fertilisation (FAO *et al.*, 2015; INRA *et al.*, 2015).

Table 2. 1 Previously published values of chemical composition of selected South African fodder types reported as a percentage of dry matter (DM)

Fodder		CP	Ash	NDF	ADF	ADL	CP	CF	Ca	P	NFE
		Meissner 1995 & 1997					Bredon *et al.* 1987				
Chloris gayana (Rhodes grass)	Pasture /hay	12.5	11	69	30	4.1	6.4 - 8.6	35.6	0.28 -0.33	0.25	46.8
Eragrostis curvula (Eragrostis, weeping love grass)	Hay	3.1-4.4	3.6-7.7	77-87	36-41	6.4-10	7.2-12.8	40.1-42.6	0.11-0.23	0.10-0.15	38.7-44.5
Eragrostis tef (Teff)	Hay	5-11.7	5.1-5.3	66-75	38-49	6.0-8.2	8.6-9.4	30.4-36.6	0.37	0.17	46.5-53.6
Pennisetum clandestinum (Kikuyu)	pasture	21.9-25	12-13	60-63	26-30	4.3-4.4	11.5-18.0	29.0-32.0	0.18-0.32	0.30-0.35	37.5-42.1
Cynodon dactylon (Bermuda grass)	Pasture	8.75-17.5	6.2-8.3	52-73	30.36	4.1-6.8	12.0	23.5	0.23	0.25	44
	Hay						6.4-10.2	28.6-43.1	0.30-0.41	0.2-0.21	41.9-54.9
Medicago sativa (lucerne, alfalfa)	Pasture	15.6-26.25	7.6-10	36-50	25-41	4.8-9.6	16.9-22.5	25.8-31.7	1.53-2.30	0.27-0.31	38.5-41.6
	Hay	15-20	8.8-10	43-51	34-39	3.8-9.7	15.9-20.9	27.9-32.8	1.35-1.66	0.25-0.27	36.8-42.2
Avena sativa (Oat hay)	hay						6.8-13.8	3.1-3.6	0.3-0.33	0.27-0.35	47.6-49.3
Veld- Sour	Grazing						6.7-8.9	32.0-36.0	0.20-0.23	0.15-0.20	44.3-46.6
Veld / redgrass	Hay	3.1-5	5.3-7.1	79-87	47-52	6.3-8.5	3.9-6.9	3.7-4.3	0.17-0.24	0.14-0.2	43.5-45.1
Veld -Sweet	Grazing						6.2-10.1	30.1-36.3	0.23-0.28	0.20-0.23	44.9-46.0

Eragrostis tef (teff) is a summer growing, annual tufted grass originating from Ethiopia where it is used for seed production. Hay cultivars have been developed and teff is a common hay fed to horses in SA (Iwanowski, 1987; Van Dyk and Neser, 2000). Feedipedia (2015) lists CP content between 8.8 – 10.5% while Bredon *et al.* (1987) gives values of 8.6 – 9.4%. When cut in late heading stage, teff showed lower CP, K, Fe and Mn content compared to those from early boot cuttings while NSC content increased and horses showed a preference for earlier cuttings over later cut hay (Staniar *et al.*, 2010). Digestibility of DM, CP, ADF and NDF dropped with the later cuttings while carbohydrate digestibility in the horse increased with maturity (Staniar *et al.*, 2010). McCown *et al.* (2011) and Staniar *et al.* (2010) found horses preferred earlier cuttings of teff which had lower NDF and ADF content.

Bermuda grass (*Cynodon dactylon)* is a hardy, creeping, tropical grass capable of withstanding heavy grazing and drought. It is well known in the horse industry in the USA where it is available as hay or is cubed and pelleted (INRA *et al.*, 2015). It is a lesser known species in SA although it is available in certain areas as hay. Protein content drops with maturity from 20% in young fertilised stands to 3 – 9 % in older hays and nutrient quality is highest in the leaves compared to the fibrous stems (FAO *et al.*, 2015). Intakes of 1.7 – 2.1 % of BW have been recorded in horses with digestibility of 39 – 53% (LaCasha *et al.*, 1999; Sponheimer *et al.*, 2003; Eckert *et al.*, 2010).

Veld grass hays are harvested from naturally occurring grasslands providing horses with one of the few hays containing a multiple species mix. Grasslands from which the hay is harvested may range from pristine through various stages of degradation which affects the quality and nutritional status of the hay (Meissner, 1997). Generally veld grasses are not fertilised or managed in any way, with reliance on natural rainfall and soil fertility to provide grass nutrients. Veld hay is at best of moderate to low nutritional value at time of cutting (Bredon *et al.*, 1987). Protein content drops significantly with age and depends on the month of harvest with February cuttings containing 6.9%, March - 5.1% and April/May - 3.9% CP while crude fibre values rise from 3.7, 4.0 and 4.3% respectively (Bredon *et al.*, 1987).

Oat-hay (*Avena sativa)* is a commonly used fodder for horses (Van Dyk and Neser, 2000) in the Western Cape region where it is grown in the winter rainfall areas and grass hays are difficult to obtain. As with other hay, time of harvest, climate and soil conditions can influence the quality. CP content dropped from 12% at ear formation to 6.3 % at dough stage in French studies with NDF values rising between ear formation and flowering and dropping again at dough stage (INRA *et al.*, 2015). Starch content can vary from 1 - 25% depending on the stage of grain formation and horses may eat the oat grain and leave the straw, in hay with mature seeds (INRA *et al.*, 2015).

In SA, lucerne is the primary leguminous fodder cultivated for hay and tends to be grown in the drier western and central parts of the country (Meissner, 1997) where it forms the basis of many horse rations where grass hays are scarce. Lucerne hays contain higher protein, DE and Ca content with lower NDF and hemicellulose content than grass hay. Lucerne generally contains higher lignin content than grass hays and coupled with increased phenolics, leads to an increase in cation exchange capacity and give lucerne a higher buffering capacity for stomach ulcers than grass (Virkajarvi *et al.*, 2012). The Ca in lucerne is considered to be the most bioavailable of any source (Hintz *et al.*, 1984; NRC, 2007; Kellon, 2008a).

Rhodes grass (*Chloris gayana*) and Smuts finger grass (*Digiteria eriantha*) are subtropical grasses grown in the Highveld regions of SA as hay species (Meissner, 1997) and are used for horses in these areas.

2.1.2 South African grazing / pasture species

Grazing is seldom available to horses in intensively managed production systems but for horses in the farming and small holding areas of SA, grazing can form a major part of their daily fodder intake. These horses range from having 100% of their ration made up of grazing from pastures or veld to stabled sport or recreational horses which are fed hay and stabled at night which are provided with grazing for limited parts of the day.

Mixed pastures of certain indigenous grasses with or without exotic species are used as grazing for many horses in SA. In the warmer, frost-free areas, grass tends to have higher CP and DE values and is termed sweetveld grass while sourveld grass occurs in the colder areas and has lower CP and nutrient content especially in the colder, drier times of the year (Meissner, 1997). Many horses with cultivated pasture access, graze kikuyu-based pastures and improved pastures of other grasses are more common on breeding farms and are seldom available to horses unless they are grazing with cattle or sheep on farms.

Kikuyu (*Pennisetum clandestinum)* is a tropical creeping grass that is often used as a pasture species for horses in SA (INRA *et al.*, 2015). It withstands high grazing pressures and is able to grow under stressful and less than optimal habitats (Marais, 2001). It responds well to fertilisation and irrigation with yields of 9 - 30t DM/Ha (INRA *et al.*, 2015) although quality varies vastly with season and growing conditions (Marais, 2001; Dugmore, 2011). Protein content in kikuyu ranges from 8.5% in the colder drier times of the year up to 25.6% CP DM in the main growing season. Increasing the rate of N fertilisation results in increased CP content and reduced NSC content of kikuyu (Dugmore, 2011). Young leaves have highest CP content which decline rapidly to below 10% within 12 weeks (FAO *et al.*, 2015). NDF content of kikuyu is lower than other tropical grasses (58 – 74% DM) and digestibility declines with increased NDF *(*INRA *et al., 2015).* Kikuyu intakes of 8.3 kg fresh weight with a digestibility of 54% have been shown for horses (INRA *et al.*, 2015). Mineral content changes through the growing season and P content drops with dormancy while Ca content increases (FAO *et al.*, 2015).

A large proportion (95%) of Ca in kikuyu is in the Ca oxalate form (Marais, 1990; in Meissner, 1997) which is largely insoluble in the alkali portions of the small intestine (SI) making the Ca unavailable to the horse (Stewart *et al.*, 2010). Ca and P availability is impaired in grasses containing 0.5% oxalate (Blaney *et al.*, 1981; Marais, 1997; Pagan, 2000). Though other authors give 20% availability of Ca from oxalate rich species, they do state higher oxalate content can reduce this amount and pastures with 0.5% oxalate have the potential to cause nutritional secondary hyperparathyroidism – a Ca deficiency state - in horses (Gartner *et al.*, 1981; Stewart *et al.*, 2010; INRA *et al.*, 2015). Kellon (2008a) reported lowered Mg content in horses grazing kikuyu pastures in Hawaii while (McKenzie *et al.*, 1981a) reported no changes in Mg balance occurred when horses ate hay with 0.5% oxalate. The possibility that oxalates bind Mg and Zn has been suggested (Hintz *et al.*, 1984).

Paspalum species, Bermuda grass, cocksfoot (*Dactylis glomerata*) and rye grass (*Lolium perenia*) are less common pasture species available to horses in the farming areas.

2.2 Concentrate feeding

2.2.1 Feeding practices

The majority of working and recreational horses in SA are fed up to 50 - 80% of their ration in concentrate feeds. If poor performance is perceived to occur in the horse, the current practice is to increase the feed portion of the ration with little cognisance of the actual reasons affecting performance (Young, 2011) or the dietary under or overprovisions which may be causing the lack of performance.

SA has many horse feed producers not all of which are registered. Some of the better known feed companies are listed in Table 2.2, with their respective market contribution. Feeds range from growth or breeder diets through to maintenance and working diets either with or without grain, endurance feeds, racing feeds and more recently the balancer and fibre feeds. Differences in feeds are said to be due to protein content, energy content and form of energy contained in the feeds. Mineral content changes in the different feeds and certain feeds in the more expensive ranges contain organic or chelated minerals.

Table 2. 2 Major South African horse feed companies with the numbers of feeds produced by each and their respective web addresses (taken from feed company websites, July 2015)

COMPANY	NUMBER OF FEEDS PRODUCED (105)	WEBSITE
ALZU	5	www.alzu.co.za
CAPSTONE	9	www.capstonehorsefeed.com
EPOL	18	www.epolequine.co.za
EQUIFEEDS	21	www.equifeeds.co.za
EQUUS	8	www.equusfeeds.co.za
GROENVOER	4	www.groenvoer.co.za
ROMIX	7	www.romixfeeds.co.za
ROYAL /VITALINE	13	www.royalfeeds.co.za
SPURWING	15	www.spurwinghorsefeed.co.za
VUMA	5	Unavailable

In SA feeds are sold on basis of crude protein (CP) content with levels ranging from 9 -25% depending on feed type (Table 2.3) and the target market although there is little basis in the literature for using CP as a formulation criteria. Young (2011) found poor correlation between advertised CP content and actual CP in feeds produced in SA.

Table 2. 3 Basic description of feeds available in the South African horse feed market (adapted from Young, 2011)

FEED	BASIC DESCRIPTION	PROTEIN %
STUD	Broodmare/ growth	14,16
RIDING	Grain	9,10,12,13,14
	Grain free / cool	10, 11, 12,14,15
RACE		12,14,16
FIBRE	Conditioners/ fodder replacers	9,10, 14,15
VETERAN	Veteran / Golden	13,14
SUPPLEMENTS	Balancer	20, 25

2.2.2 Feed presentation

Feeds can be bought as meals, cubes/nuts of 6 - 8mm diameter with 12mm length, pellets 4mm diameter and muesli's/ course mixes. Nuts and pellets are compound feeds that are ground and cubed or pelleted ensuring homogenous nutrient content and longer shelf life than other feeds (Frape, 2010). Horses showed no preference for harder nuts although they did not like softer, crumbly ones (van der Merwe, 1975; in Frape, 2010). The more recent addition of muesli feeds to many company lines is due partly to the preference of horses in heavy work for larger quantities of muesli type feeds than meals, cubes/ nuts. Cubes have lower bulk and longer shelf-life than mueslis and are easier to produce (Frape, 2010). Pony mares with *ad libitum* access to pelleted vs muesli type feeds ate slower, had a lower DE intake and ate less frequently when offered muesli compared to pellets (Argo, 2001). Horses showed a preference for high fibre feeds compared to low fibre ones (Goodwin *et al.* 2002; in Frape, 2010)

The processing method used in feed production can influence the glycaemic and insulin responses in horses, and raw material selection and processing methods need to be taken into account when horses susceptible to insulin insensitivity are being fed (Nielsen *et al.*, 2010). Depending on raw material selection a lower glycaemic response is produced from muesli type feeds which have a slower intake rate compared to pelleted feeds which are finely ground before pelleting and are therefore digested faster raising insulin levels faster (Frape, 2010).

2.2.3 Raw material selection

In SA there are no legal requirements governing the ingredients used in horses feeds and the only legal obligation companies have as per "The Fertilisers, Farm Feed, Agricultural Remedies and Stock Remedies Act 36 of 1947 "is to specify levels of CF, CP and the Ca:P ratio in the feed (Young, 2011). The use of raw materials for horse feeds is therefore left to the company's discretion and various ingredients are used as shown in Table 2.4. Various processes are used to change the site of digestion and the digestibility of the feed ingredients such as micronisation, extrusion and crushing (Julliand *et al.*, 2006). The selection of raw materials has become something of a company hook where either the type of raw material (e.g. copra meal or full fat soya) or the processing of it (e.g. micronisation and extrusion) become the company signature. The physical presentation (muesli, meal, cube or pellet) also becomes somewhat distinguishing per company.

Table 2. 4 Some commonly used raw materials in South African horse feeds as per feed labels and company websites

NUTRIENT	RAW MATERIAL
CARBOHYDRATE	Maize, wheaten bran, hominy chop, sugar cane molasses, oats, barley, extruded maize, crushed raw maize, bran middling's and rice bran.
PROTEIN	sunflower oil cake, soya oil cake, full fat soya, plant protein products, sunflower meal, lupins, canola meal, lysine, methionine
FIBRE	Lucerne, oat-hay
MINERALS	Salt, feed lime / limestone, mono-calcium phosphate, di-calcium phosphate, , magnesium, manganese, iron, zinc, copper, cobalt, iodine, selenium, chromium, potassium,

Regardless of raw material selection, feed companies are still under obligation to provide nutrients in the combination of raw materials they provide. While raw material formulations may be proprietary, the methodology behind the feed formulations should be standard to allow the provision of nutrients in accordance with horse requirements. Figure 2.1 shows how the animal's genotype and environment affect their dry matter intake (DMI) (Emmans and Kyriazakis, 2001). This should be used to generate metabolic models for the various horse types to inform the dry matter intake across a range of different horse management systems and across various breeds of horses and form the basis of horse feed formulation.

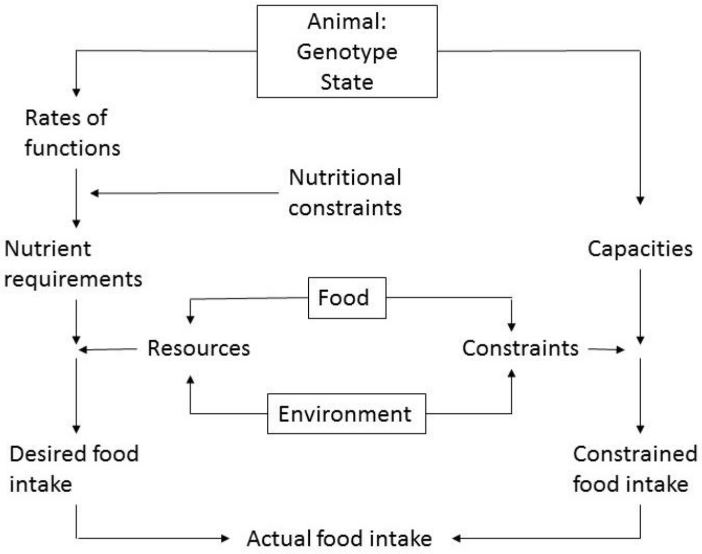


Figure 2. 1 A schematic representation of the theory that 'animals eat to meet their requirements subject to constraints' (Emmans and Kyriazakis, 2001)

Raw material selection allows manufacturers to choose feed ingredients to provide various nutrients to horses and these can be differentiated into groups providing the following macro-nutrients: carbohydrates, protein or fibre. Feed manufacturers need to take cognisance of the mineral levels in the raw materials when formulating a ration.

2.2.4 Carbohydrates

Carbohydrates in the form of starches and sugars provide energy to the horse via enzymatic digestion in the SI which contribute to the glycaemic and insulin response of the horse to a feed. Hoffman (2009) differentiates carbohydrates into fermentable fibres (e.g. fructans and hemicellulose) derived from fodder or hydrolysable starches which are digested in the SI such as starch, hexoses, and certain disaccharides. SI capacity to digest starch is limited and needs to be restricted to under 0.35 - 0.4% of intake but the form in which the starch is provided may lower this (Hoffman, 2009). If starch is not digested in the SI and reaches the hindgut, it ferments rapidly and may disrupt the microbe populations putting the horse at risk of colic and laminitis (Hoffman, 2009). The form of carbohydrate provided will determine the amount and type of work a horse may perform with starches providing glucose and glycogen which is efficient in fuelling speed work while fermentable carbohydrates or fibres provide a slower energy source via volatile fatty acid production in the hindgut (Julliand *et al.*, 2006; Hoffman, 2009).

The source and processing method of grains will determine their SI digestibility with whole oats having the highest digestibility while maize and barley are resistant to SI digestion unless they are processed in some way (Julliand *et al.*, 2008). Brans (e.g. wheat and rice) and hominy-chop are by-products of the food industry often used in stock feeds – they are high in fibre and contain intermediate levels of carbohydrates. Julliand *et al.* (2008) suggested the reduction of starch in race and sport horse rations would optimise digestion and metabolic use of nutrients and could play a role in reducing colic in these horses.

Of the carbohydrate sources mentioned in Table 2.4 all but molasses are derived from cereal grains and will contain phytic P which has limited availability to the horse and if fed in excess can reduce the absorption of Ca and other cations from the diet (van Doorn *et al.*, 2004a; Hintz, 2009; Frape, 2010; Stewart *et al.*, 2010; Fowler, 2013).

2.2.5 Protein

The quantity of CP from concentrated feed and fodder and the proportion of these in the ration, as well as the carbon to nitrogen balance (C:N) influence the usefulness of CP to horses. CP requirements are affected by the protein quality of feed and fodder and increase with pregnancy, lactation, growth and work rate in horses. CP quality can be described by the amino acid profile and SI absorption of the CP fraction (Virkajarvi *et al.*, 2012). Muscle tissue development and growth are limited by amino acid availability (Coenen, 2008) and the NRC (2007) makes provision for increased CP requirements for work of 0.35 g CP / kg BW.

Protein requirements of horses can vary from 6 – 16% of total DMI depending on growth, breeding and exercise status of the horse (Siciliano, 2015). With increasing feed quantities supplied to provide energy for increased workloads, the CP intake increases to above maintenance levels (Coenen, 2008).

Both under and overprovision of CP in horse rations have consequences to the horse and should be avoided. Overprovision of CP in rations has been shown to increase race times (Glade, 1983; in Young, 2011), increase water intake and urine output and results in ammonia production in stables which may cause respiratory distress (Nielsen, 2001; in Young, 2011). As the fibre content of fodder increases it has a negative impact on protein digestion and reduces the quality of the protein in the fodder (Kellon, 2008a; Longland, 2012). Many of the protein feeds are by products of the oil industry and chemically extracted seed meals have lower oil components than expeller meals (Frape, 2010).

Protein seed meals of soya and sunflower commonly used in SA, typically have high P and low Ca levels with varying amounts of other minerals depending on their parent grain, source and processing method. Soya based proteins have an amino acid profile well suited to a horse's needs while sunflower oil cake lacks sufficient lysine for horses although methionine and cysteine levels are high (Frape, 2010; Kellon, 2013). Phytates present in soya products limit Zn availability in humans and has been shown to occur in pre-ruminant calves fed soya protein in milk replacers (Suttle, 2010; Basnet *et al.*, 2014). No research has been conducted in horses to ascertain whether reduction in Zn absorption occurs with soya based feeds. The processing method and end product used in feeds may also affect availability of Zn from soya products as shown in rats with reduced absorption from isolates and soy concentrates compared to full fat soya (Lönnerdal, 2000). Copra meal (a by-product of the coconut industry) has recently become available in SA for horse feed. It contains poorly bioavailable protein of low quality (Frape, 2010) , the Ca:P ratio is inverted and it has a high Fe content.

Lupins, peas and individual amino acids (usually DL-methionine and lysine-HCl) may be added to feeds to increase protein content although not in large quantities.

2.2.6 Fibre

To increase the fibre content of feeds, the addition of chaffed lucerne, oat-hay and teff have been used but recently more emphasis is being placed on alternatives sources of fibre for horses and new products such as soya hulls and beet pulp have been added to feeds as a fibre component. Historically whole oats, chaff and hay diets contained sufficient fibre which assisted the horse in maintaining gut health. However with today's shift towards more processed, higher energy, low fibre feedstuffs and decreased fodder intakes, horses are more likely to suffer from colic, laminitis and are at higher risk of developing stereotypical behaviours (Bulmer *et al.*, 2015).

Horses exhibit a preference for high fibre diets when offered the choice and are motivated to seek out hay and spend more time searching for food when fed a low fibre compared to a high fibre diet (Elia *et al.*, 2010). Fibre sources used in feeds tend to contain differing levels of Ca and P and those derived from grains will contain phytates which may limit Ca absorption if fed in excess. Beet products have a very high Ca:P ratio and tend to have high Fe and low Zn and Cu content requiring supplementation to correct this. Soya hulls are a recent addition to the fibre choice in SA and their Fe content is high.

2.3 Minerals

Horses rely on dietary intake of feed, fodder, water and soil to supply minerals. That there is a proliferation of feed supplements, each with a different mineral content, indicates that whole feeds are somehow not predictably performing their function to either the horse or the human's satisfaction.

A positive mineral balance occurs when a horse retains more of a mineral in the body than it is losing via endogenous losses and generally describes growing horses or those being provided with excess minerals in the feed. Negative balance occurs when the horse is using body stores to compensate for a lack of dietary intake and will result in a deficiency in the long-term while zero balance is a state where intake equals endogenous losses with no retention occurring and the horse is in a state of homeostasis (NRC, 2007).

Dietary excesses or positive mineral balance will result in storage of minerals in body tissues such as the liver for minerals such as Cu, Fe, Mn and Zn or bones for Ca, P and Mg, while a deficiency will result in the utilisation of these body stores in order to maintain bodily functions as far as possible. A state of maintenance in which intake equals requirements should be sought to eliminate wastage of nutrients and potential environmental contamination of minerals such as P and Cu (Suttle, 2010).

In horses, minerals are eliminated via urine or faeces and they are one of the few mammals which can absorb Ca in higher quantities than required and eliminate it through the urine if it is excess to bodily needs (Kienzle and Zorn, 2006; Clauss *et al.*, 2007; Kellon, 2008a). Regulation of body levels of Ca, Mg and P are maintained by hormonal control and absorption or elimination via the renal pathways can change to meet needs when deficient or excess states are found (Kellon, 2008a; Frape, 2010). Excess Cu and Mn are excreted via bile in the faeces while Zn is excreted, via pancreatic secretions, in the faeces and limited renal excretion occurs for these trace minerals (Wagner *et al.*, 2005; Suttle, 2010). No mechanism exists for disposal of excess Fe from the body. It is stored as ferritin in the liver and spleen (Jackson, 2000b; Kellon, 2008a; Vervuert, 2008; Suttle, 2010) and excess Fe can cause liver damage (Casteel, 2001).

Compared to production animals horse nutrition and particularly research into mineral nutrition of horses is still in its infancy and many of the nutrient interactions and bioavailability's found in other species are not well investigated in horses (Kienzle and Zorn, 2006). Horses have proven time and again that principles applied to other species cannot always be taken at face value when applied to them (Kienzle and Zorn, 2006).

2.3.1 Mineral sources

Minerals missing from the feed and the fodder can be provided by pure mineral sources, inorganic or organic, or inadvertently in the raw materials themselves. In addition to the absolute amounts of each mineral being provided, there is the ratio between minerals which is also critical (NRC, 2007).

Phytic acid forms 59 - 70% of the P in cereal grains and 20 - 46% in legume seeds and reduces intestinal absorption of Ca, P, Fe, Mn and Zn (Vervuert, 2008). As P is absorbed in the lower portion of the large intestine, caecal fermentation by microbes increases P availability from phytic acid (Vervuert, 2008).

It is generally accepted that supplementary sources of minerals are more available than plant based ones, with limestone, di-calcium phosphate and mono-sodium phosphate being highly available to horses (Hintz and Schryver, 1972; Vervuert, 2008). Oxides, carbonates, sulphates and chlorides are commonly used forms of inorganic minerals used in horse rations (Frape, 2010). Fe contamination of mineral sources, such as di-calcium phosphate and magnesium oxide, used to supplement rations may add a significant amount of Fe to feeds and supplements (Suttle, 2010).

2.3.2 Inorganic and organic mineral sources

Organic minerals refer to a group of manufactured products where, through chemical processes, mineral elements are bonded to organic molecules such as amino acids or polysaccharides (Cohen and Steward, 2014). The form and number of the bonds between the mineral and the carrier will determine the site of digestion and availability of that mineral to the animal (Kellon, 2008a).

Wagner (2005) found no differences in absorption and retention of Cu, Mn and Zn from oxide, sulphate and chelated forms of the minerals and there was no change in liver or hoof wall content of these minerals when horses received a diet of 40% hay: 60% conc. Vervuert (2008) reported that organic minerals have no clear advantage over sulphates as shown by Baker (2005) where Cu sulphate had a higher digestibility than chelated Cu. Mares supplemented with Cu sulphate showed significant increases in plasma Cu levels compared to those supplemented with proteinated Cu (Jančíková *et al.*, 2012) while Wagner *et al.* (2011) found chelated Cu had higher retention than $CuSO_4$, although all horses in this study had negative retention.

Yearling horses fed proteinated forms of Cu and Zn showed greater hoof growth and hip height compared to yearlings fed inorganic trace minerals (Ott and Asquith, 1995) although it is unclear if this effect was from the minerals or the amino acids the organic minerals were bonded to. It remains unclear as to whether organic minerals offer an advantage over inorganic forms in adult horses while in weanlings or yearlings there seems to be a slight advantage in feeding organic minerals. The differences in absorption of the minerals could be as a result of competition from other minerals (Frape, 2010). Warren (2009) replaced 100% of inorganic sulphates of Cu, Mn and Zn with organic chelates and tested the effect of hoof - wall growth, sole thickness and humoral immune function in lactating mares. They found no significant differences in hoof-wall growth and sole depth although numerical differences in sole depth occurred in the organic trace mineral group. Similarly IgG titres were not significantly different between mares supplied with organic or sulphate form of the minerals.

The cost and ligand structure of the organic minerals needs to be taken into consideration when selecting mineral forms to feed. KER recommends performance horses be fed a third of supplementary trace minerals from chelated or organic sources and two thirds from inorganic sources in rations (Jackson, 2000b).

2.3.3 Vitamin and mineral packs

Various vitamin and mineral (Vimi) packs are available for inclusion in horse rations. Feed companies typically have at least one or two Vimi packs which they include in the various rations to differentiate the higher cost products with higher nutrient inclusions from the cheaper maintenance and leisure type feeds. Customised Vimi packs are manufactured in SA and can be included in rations to allow a feed to be matched to horses needs and fodder types. Table 2.5 shows a typical equine Vimi pack designed to be included in 1t of mixed feed.

Table 2. 5 Example of a Standard Vitamin and Mineral pack used in the production of horse feeds in SA

[Formulation]:	Std Horse (1T)
[Unit size]:	2.5 kg
[Version]:	12
Vitamin A (1000 000iu)	8,000,000.0000 IU
Vitamin D3 (500 000 iu)	1,000,000.0000 IU
Vitamin E (500iu)	100,000.0000 IU
Vitamin K3 43%	3.0000 g
Vitamin B1 (Thiamine Mononitra	5.0000 g
Vitamin B2 80 % (Riboflavin)	5.0000 g
Niacin 99.5%	10.0000 g
Calcium Pantothenate (98%)	5.0000 g
Vitamin B12 1g/kg (mixed)	0.0150 g
Vitamin B6 98%(Pyrodoxine HCl)	3.0000 g
Choline (Chloride 60%)	86.5800 g
Folic Acid (96% pure)	5.0000 g
Biotin 2%	0.0200 g
Anoxytol Dry (Pet Anti-Oxidant)	125.0000 g
Manganese Sulphate 31%	14.0000 g
Zinc (So4-35% Mono)	30.0000 g
Copper (So4-25.2% Penta)	20.0000 g
Potassium Iodide (Iodine 76.45	1.0000 g
Ferrous (So4-30% Mono)	100.0000 g
Magnesium Oxide 50%	100.0000 g
Selenium (4.5%)	0.3000 g
(Carrier) Dolomite	1,189.9000 g

2.3.4 Mineral interactions

Interactions between minerals have been shown to exist in many species with synergistic or antagonistic relationships occurring between minerals. The provision of minerals in horse rations needs to be balanced in terms of the known interactions and further research may show more of these interactions to occur in horses. Currently, research on mineral interactions in horses is limited (Kienzle and Zorn, 2006).

The Ca:P ratio of the ration is critical to Ca absorption and horses can develop nutritional secondary hyperparathyroidism (Ca deficiency), despite receiving sufficient Ca in the diet, if the Ca:P ratio is under 1:1 (Stewart *et al.*, 2010). Caple (1982) studied the Ca and P levels in Australian racehorses and found 40% of the studied population had inadequate Ca:P balance in their rations. Blood levels of Ca are tightly maintained by parathyroid and calcitonin hormones and horses may show adequate serum Ca levels even when they are severely deficient (Ronen *et al.*, 1992; Sasaki *et al.*, 2005). High P intakes decrease Ca digestibility (van Doorn *et al.*, 2004b) and Schryver (1971; in Frape, 2010) reported up to 50% reduction of Ca absorption when inorganic P in the diet was increased from 2 – 12 g/kg even in the presence of adequate Ca. The addition of phytase increased Ca absorption in horses fed phytate rich diets but did not improve P digestibility. Mg remained unaffected by phytate or phytase in the diet (van Doorn *et al.*, 2004a).

Ca and Mg compete for digestion sites in the horse and Mg may form insoluble salts with P, reducing digestibility. High P content in the diet decreased Mg absorption (Westendorf and Williams, 2015) while Ca absorption can be increased by the addition of Mg to the diet (Vervuert, 2008). As of yet, no Ca:Mg or P:Mg ratios have been set by the NRC for horses but Johnson *et al.* (2004) and Kellon (2008a) suggest an optimal ratio of between 1.5 – 2 :1 for Ca:Mg in equine diets.

High Ca content in feeds have the potential to reduce Zn absorption as shown in chickens when a 3.8 fold decrease in Zn absorption occurred when Ca content was increased from 6 to 7.4 g Ca/kg (Suttle, 2010). Although this has not yet been proved to be true in horses, it would be prudent to take the Ca content into consideration when calculating Zn inclusions in a ration (Kienzle and Zorn, 2006).

Interactions between trace minerals and other nutrients are found in many species and certain of them have been shown to exist in the horse. Lawrence (1987; in Frape, 2010) showed reductions of Cu and Mn content in the spleens of horses consuming > 1400 mg Fe/kg diet while Zn was reduced at an Fe intake of 890 mg/kg. Interactions between Cu and Zn in the diet have been shown by Spais *et al.* (1978; in Cymbaluk *et al.,* 1990) where Cu absorption was decreased by Zn content of 126 mg/kg in the diet and cases of Cu deficiency were reported in horses grazing high Zn fodder (NRC, 2007). Other factors known to affect Zn uptake are high CP, Ca and Cu content of the diet (Jackson, 2000b). Cu absorption is affected by high Ca and CP content in rations (Lawrence and Pagan, 2005). Horses on predominantly legume fodder therefore need special attention to mineral balance in their feed and to ensure that the feed matches the mineral provision in the fodder (Lawrence and Pagan, 2005).

The NRC (2007) set the Cu:Zn:Mn ratios of 1:4:4 for horses while Kellon (2008a) suggests a ratio of 1:3:3 for these minerals and a ratio of 1 part Cu to 4 or 5 Zn has been suggested by Lawrence and Pagan (2005) . As of yet, Fe has not been included in NRC ratios for horses although it is known to interact and compete for absorption with Zn, Cu and Mn in the horse. Johnson *et al.* (2004) suggests ratios of between 4:1 and 10:1 for Fe:Cu and Kellon (2008a) reports improvement in insulin resistance and reduction of body Fe stores when ratios are maintained closer to 4:1 Fe:Cu. When NRC recommendations for Cu, Fe, Mn and Zn are compared to one another, the ratio between them is close to Kellon's recommendations, with a ratio of 1:4:4:4. Ferritin has an affinity for Mn, which will displace Fe from ferritin molecules exacerbating Fe overload when Mn intakes are high (Kellon, 2008a). Horses exhibiting pica had lower serum Cu and Fe content and Cu:Zn ratios than horses without pica (Aytekin *et al.*, 2011). The ideal mineral ratios are summarised in Table 2.6.

Table 2. 6 Summary of recommended mineral ratios for optimal mineral uptake and utilisation in horses sourced from the literature

MINERALS	RECOMMENDED RATIO
CA : P	1.2 - 2 : 1
CA : MG	1.5 - 2 : 1
CU : MN : ZN	1 : 4 : 4
CU : ZN	1 : 3 - 5
FE : CU	4 - 10 : 1
FE : CU : MN : ZN	4 : 1 : 4 : 4

2.3.5 Trace minerals in immunity

Trace minerals play a pivotal role in immune system function and slight deficiencies can have a large impact on the health and functionality of the immune system as shown in Figure 2.2. Cu is important in many aspects of immune function from energy production through to antibody development and replication of lymphocytes (Larson, 2005). A Cu deficiency has a direct impact on immune response and may be a reason vaccines fail (Arthington *et al.*, 2002). It reduces humoral and cell mediated immunity as well as reducing bactericidal neutrophil activity in steers (Larson, 2005).

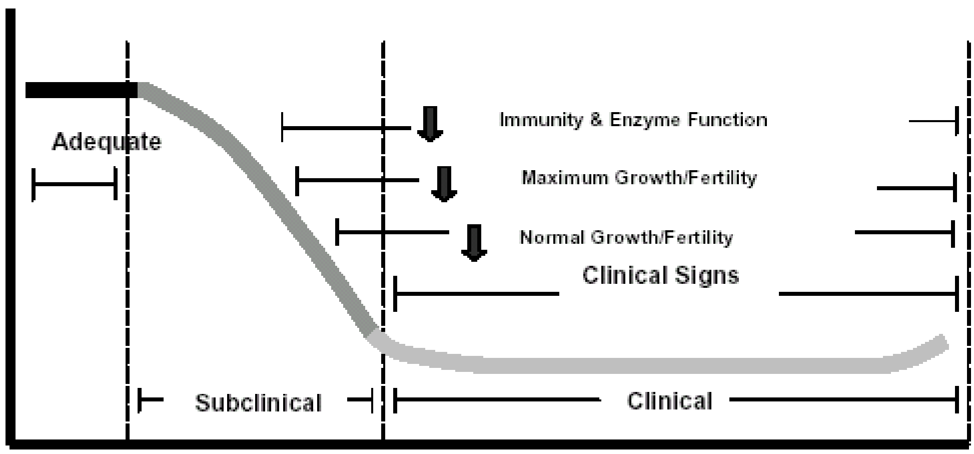


Figure 2. 2 Effect of declining trace mineral status on animal performance (Larson, 2005)

Zn is integral to immune function via its role in protein synthesis, energy production, anti-oxidant enzyme and lymphocyte production as well as antibody formation. Zn deficiency decreases the rate of cell division resulting in reduced immune function and reduction of non-specific immunity, T and B lymphocyte numbers, it suppresses delayed hypersensitivity and lowers antibody production (Basnet *et al.*, 2014). In other species, Zn downregulates inflammatory cytochrome production and may act as an anti-inflammatory (Hagmeyer *et al.*, 2015) while an increased susceptibility to viral infections has been shown to occur when Zn is deficient (Watts, 1988b). With the prevalence of African Horse Sickness and the recent increase in other equine viruses in SA and the findings of Horne (2015) showing 67% of sampled horses were deficient in Zn, correct trace mineral nutrition could help to improve immune function and anti-body production in horses. Together with Mn, Zn is integral in epithelial integrity and will be important in allowing the lining of the lungs, respiratory and digestive tract, reproductive system to withstand pathogenic infiltration (Larson, 2005).

Immune impairment due to Fe deficiency is unlikely to occur in horses as no natural Fe deficiency is experienced in equines (Kellon, 2008a; Frape, 2010). Excess Fe can negatively impact the immune system as in the initial stages of immune response to a pathogen the body sequesters Fe in an effort to starve pathogens of Fe they need for growth and replication (Weinberg, 1978b; Watts, 1988a). If the Fe storage mechanisms are overwhelmed, circulating Fe is available for growth of pathogens allowing disease to develop in the animal (Weinberg, 1978a; Watts, 1988a, 1989). The balance between Cu, Fe and Zn in the body is important to immune function and excess or relative deficiency of any of these three can lead to susceptibility to pathogenic attack (Watts, 1988a, 1989).

2.4 Horse requirements

The latest nutrient requirements set out by the NRC (2007) provide work and maintenance classes to encompass most types of domestic horses, using heart rate and exercise intensity to accurately calculate work categories and breeding or growth categories for stud animals.

These categories vary in their nutrient needs and cognisance of these variations needs to be taken when formulating rations for horses (Young, 2011). Breeding and growth status, as well as special nutritional needs are also accounted for with differing nutrient recommendations for the various life stages and for certain disease states.

Major mineral levels are given as set requirements while many of the trace mineral levels are given as recommendations as the exact daily intakes are not yet determined (NRC, 2007). As the guidelines stand, there is still debate as to whether the levels of minerals suggested are sufficient to meet the growth needs of horses in training (Frape, 2010). Due to mineral interactions and unknown mineral availability, KER set recommendations higher than those given by the NRC (2007) in an attempt to account for unknown variables that may affect absorption and utilisation of nutrients (Pagan, 2009) (Table 2.7).

Table 2. 7 Kentucky Equine Research unit recommendations as multiples of NRC (1989) requirements (Pagan, 2009)

Nutrient	**Class of Horse**				
	Maintenance	Pregnancy	Lactation	Performance	Growth
Protein	1.1	1.1	1.0	0.75 - 0.9	1.0
Energy	1.0	1.1	1.0	1.0	1.0
Macrominerals	1.2 - 1.3	1.1 - 1.9	1.1 - 2.3	0.8 - 1.9	1.3 - 3.1
Microminerals	1.0 - 2.3	1.4 - 2.6	1.4 - 2.7	1.0 - 2.4	1.4 - 2.6

2.4.1 Energy

Horses in work cannot support their requirements on the intake of fodder alone. Consequently, energy density of feeds will often increase as the work load of a horse increases and work rations contain more digestible energy (DE), higher CP and better quality feed ingredients than maintenance or leisure type ones. In addition to work requirements, higher feeding rates can increase DE requirements by 10 – 12% due to the higher metabolic rate of the liver and gut (Martin-Rosset, 2008). Light breeds require approximately 20% more DE for maintenance than heavy breeds while ponies using 10 – 15 % less energy than light breeds (Martin-Rosset, 2008). Both a positive or negative energy balance will result in weight loss or gain and BCS can be used to determine the energy intake of a horse (Carroll and Huntington, 1988; NRC, 2007).

Voluntary feed intake theory is not well developed for the horse (Young, 2011). It would appear that a horse will generally eat to meet or exceed their DE requirements. Ponies fed increasing amounts of sawdust in a mixed grain ration maintained their DE intake up to the 25% sawdust inclusion by increasing intake (Laut *et al.*, 1985) where constraints to feed intake obviously occur at this point (Emmans and Oldham, 1988). Argo (2001; in NRC, 2007) reported that ponies fed *ad libitum* displayed DE intakes higher than requirement and gained weight for 6 - 8 weeks before restricting their intake and maintaining their weight. Intake is usually stimulated by the inclusion of sweet ingredients such as molasses (Hill, 2007) and feed intake has a marked impact on concomitant intake of minerals.

Energy requirements in the working horse will increase due to the higher energy cost of working muscles, the respiratory and cardiovascular system and increased muscle tone (Martin-Rosset, 2008). Table 2.8 outlines the NRC (2007) work category descriptions. Furthermore, the NRC (2007) gives work expenditure as 110 - 140% of maintenance values while the European recommendations are 125 – 200% of maintenance energy levels (Martin-Rosset, 2008).

Table 2. 8 Description of weekly workloads of horses in the light, moderate, heavy and very heavy exercise categories (NRC, 2007)

Exercise category	Mean Heart Rate (beats/min)	Description	Types of events
Light	80	1 to 3 hours per week; 40% walk, 50% trot, 10% canter	Recreational riding, beginning of training programmes, show horses (occasional)
Moderate	90	3 to 5 hours per week; 30% walk, 50% trot, 10% canter, 5% low jumping, cutting or other skill work	School horses, recreational riding, Beginning of training/breaking, show horses (frequent), polo, ranch work
Heavy	110	4 to 5 hours per week; 20% walk, 50% trot, 15% canter, 15% gallop, jumping or other skill work	Ranch work, polo, Show horses (frequent strenuous events), low-medium eventing, race training (middle stages)
Very Heavy	110 to 150	From 1 hour per week very high speed work to 6 to 12 hours per week slow work. This category includes Quarter Horse, Thoroughbred and Standardbred racing, Endurance, Upper Level 3-day eventing	Racing (quarter horse, Thoroughbred, Standard-bred, endurance), Elite 3-day event

In the latest German requirements mentioned by Coenen *et al.* (2011), body composition, physiological state, breed variations, work level, housing and weather can affect maintenance energy requirements (Table 2.9). Ponies usually need less energy per kg BW than Thoroughbreds which have a higher metabolic rate. Cold, wet conditions may increase maintenance energy requirements by 10 - 20% while overweight horses may require 15% less energy than those with moderate body condition (Coenen *et al.*, 2011). Stallion maintenance requirements are 10 – 15% higher than a gelding or mare of the same size (Martin-Rosset, 2008).

Table 2. 9 Mean maintenance energy requirements of horses in ideal body condition and moderate training stage, stabled in a box (Coenen *et al.,* 2011)

Breed	Maintenance energy requirements (MJ ME/kg $BW^{0.75}$)
Warmblood	0.52
Thoroughbred	0.64
Ponies	0.4
Other breeds	0.5 - 0.5

2.4.2 Protein

Protein is one of the most abundant substances in the body and is present in muscles, hormones, RNA and DNA haemoglobin, cell receptors, antibodies and cytokines. Protein digestion occurs in the SI with little to no absorption of microbial protein in the hindgut (NRC, 2007; Frape, 2010; Coenen *et al.*, 2011). Protein requirements are affected by protein quality or amino acid content of a feed, the protein digestibility and horse factors such as exercise, pregnancy, growth and lactation. Daily minimum maintenance CP requirements can be calculated as BW (kg) x 1.08 = g CP/kg BW, with BW (kg) x 1.26 giving average maintenance intake while elevated maintenance requirements are given using BW (kg) x 1.44 (NRC, 2007). Muscle tissue development and growth is limited by amino acid availability (Coenen, 2008) and NRC (2007) makes provision for increased CP requirement for work of 0.35 g CP / kg BW.

Optimum microbial fermentation relies on the balance between fermentable carbohydrates and nitrogen in the diet and cannot be ignored when providing rations for horses. At present rations providing excess CP to horses increase deamination in the hindgut and burden the liver with excess NH_3 which it converts urea. This may have detrimental effects in the horse (Young, 2011). Nutrient synchrony in terms of C:N provision in horses needs to be investigated, and is linked to caecal and microbial health, as well as long-term organic acid absorption (Young, 2011).

Protein requirements of horses can vary from 6 - 16% depending on the work and growth stage (Siciliano, 2015). With increasing feed quantities supplied to provide energy for working horses, the CP intake increases to above maintenance levels (Coenen, 2008). Protein is made up of amino acids and a horse's requirements for protein would be more accurately described as amino acid requirements (which besides for lysine) have not been determined for horses (NRC, 2007). Therefore it is necessary to supply horses with adequate amounts of high quality CP in the diet to allow sufficient amino-acid provision for maintenance functions and production of tissues, enzymes and hormones (Siciliano, 2015).

The oversupply of protein is unwanted in race and sport horses due to increased water intake, higher levels of heat and metabolic stress and longer times to completion experienced as well as the increased urea elimination in stables which can affect the respiratory system (Coenen, 2008). Young (2011) found protein deficiency in sport horses is rare in SA. No actual recommendations are set for working horses as far as CP provisions go but the upper safe level is assumed to be 2 g digestible CP per kg BW (Coenen, 2008). Horses may benefit from higher protein intakes the day after intense exercise to enable tissue repair (Young, 2011).

2.4.3 Major Minerals

2.4.3.1 Calcium

Daily Ca levels for horses at maintenance are calculated using endogenous losses from work done by Schryver *et al.* (1970) and Buchholz-Bryant *et al.* (2001), with the latest NRC recommendation remaining unchanged at 20 mg/kg BW (NRC, 2007) or 0.043 g Ca/kg BW. Broodmares require extra Ca during late gestation and lactation to meet increased needs due to foetal growth and milk production. Foetal growth in the last trimester of pregnancy raises Ca requirements with the NRC (2007) setting requirements at 0.056 g Ca / kg BW for months seven and eight while 0.072 g Ca / kg BW is required for the last three months (NRC, 2007). Lactation Ca requirements vary with time after foaling. Mares in early lactation (0 - 3 months) require [(0.04 g x kg BW) + (0.032 x kg BW x 2.4 g)] Ca which drops to [(0.04 g x kg BW) + (0.026 x kg BW x 1.6 g)] for months 4 - 5 and months 5 on [(0.04 g x kg BW) + (0.020 x kg BW x 1.6 g)]. Although excess Ca provision appears not to have a negative effect, as shown in Shetland foals fed 25 g/kg Ca for 4 years by Jordan *et al.* (1975), other work shows excess Ca intake will also increase P excretion and can decrease absorption of Fe, Mg and Mn (Frape, 2010). Ca absorption of 71% in six month old foals dropped to 42% by the time the horses reached 24 months of age (Frape, 2010) and NaCl inclusions of 3 - 3 5% increased absorption and retention of Ca (Schryver *et al.*, 1987).

Bone density changes in exercised horses should increase the need for Ca with periods of idleness and type of work having a marked effect on bone density (Buchholz-Bryant *et al.*, 2001). Speed work with short sprints increases bone density more than draft or endurance work while stall rest decreases bone mineral density (Buchholz-Bryant *et al.*, 2001; Mansell *et al.*, 2001; Pipkin *et al.*, 2001; NRC, 2007). As dietary Ca increases, there is an increase in retention which would most likely result in skeletal increases of this mineral (Vervuert, 2008). Ionised Ca^{2+} levels in the blood drop after exercise and higher content of Ca in the diet may be able to offset this drop as Vervuert (2006) found increased blood Ca^{2+} levels when horses were fed 96 g of Ca and 56 g of P representing 200% and 300% of NRC requirements respectively. No negative impact of this high feeding rate was observed on parathyroid levels which would indicate they are tolerated well by horses. Feeding Ca at levels 275% higher than NRC (1989) recommendation resulted in increased retention and may have positive effects on bone remodelling and resorption in horses (Buchholz-Bryant *et al.*, 2001). Bone resorption and mineralisation differs with age of the horse and it may be prudent to alter dietary Ca provisions to optimise this process in horses returning to work after a period of idleness (Buchholz-Bryant *et al.*, 2001; Mansell *et al.*, 2001; Pipkin *et al.*, 2001). Carbonate, sulphate and oxides are commonly used Ca salts for horses and supplementation as di-calcium phosphate is a reliable source of Ca for horses (Frape, 2010). Chelated Ca was absorbed at a similar rate to $CaCO_3$ (Highfill, 2005; in Frape, 2010). Excess Ca intakes are easily excreted in the urine by horses and Shetland foals fed diets with 25 g Ca /kg DM for 4 years with a Ca:P ratio of 6:1 had slight bone changes compared to those fed normal dietary levels (Frape, 2010). Skeletal growth, maintenance and bone remodelling requirements all need to be taken into consideration when providing Ca for growing horses and growing horses in training which will benefit from Ca intakes in excess of NRC (1989) recommendations as shown by (Nielsen *et al.*, 1998; Stephens *et al.*, 2004)

2.4.3.2 Phosphorous

P is absorbed from the large intestine although diet type can change absorption areas. Fodder based diets permit for no proximal SI absorption of P, while in horses fed only concentrate, the absorption occurs in the distal SI. A net excretion of P occurs in the caecum and ventral colon with most of the absorption occurring in the small colon (Frape, 2010). Di-calcium phosphate and bone meal are reliable sources of P in horses with a digestibility of 45 - 50% (Frape, 2010) although Fe content of these products may be high and contribute significantly to Fe intake (Suttle, 2010). P absorption drops from 52 to 6% between 6 and 24 months age (Frape, 2010). P absorption and retention was enhanced by dietary NaCl inclusions of 3 – 5% (Schryver *et al.*, 1987).

Grain based diets which are high in P are very palatable, making domestic horses receiving unfortified grains more susceptible to Ca deficiencies than their wild counterparts with limited access to grains. Phytic P found in grain and seeds is 35% available to horses and with the addition of Ca and often P to grain based feeds, horses are less likely to experience deficiencies in Ca and P (Frape, 2010).

Faecal P excretion increases with dietary provision and needs to be balanced in terms of environmental risk factors (Frape, 2010; Westendorf and Williams, 2015). Parvage *et al.* (2015) found P run-off increased 18 fold in one year from intensively managed horse camps where no manure removal occurred. NRC (2007) P requirements for maintenance have remained unchanged at 0.028 g P/kg BW due to concern over environmental pollution although, studies show 0.034 g P/kg BW may be a better estimate of requirement and the absorbability of the P can be an important consideration in P provision (NRC, 2007).

Requirements for growth are set at 17.8 g P per kg of gain over the maintenance requirements (NRC, 2007) and like with Ca, young, growing horses in training need a minimum of 66 mg P/ kg BW (Nolan *et al.*, 2001). Ca and P nutrition of the pregnant mare has also been shown to affect foal size and bone thickness (Lepeule *et al.*, 2013).

2.4.3.3 Magnesium

The absorption of Mg occurs in the distal SI with a small amounts absorbed from the large intestine (Frape, 2010). Mg homeostasis in horses at rest or in exercise appears to be controlled via gut absorption and renal excretion (Vervuert, 2008; Frape, 2010). McKenzie *et al.* (1981b) showed Mg absorption was not affected by the presence of oxalates while Kellon (2008a) reported low ionized Mg levels in horses grazing kikuyu pastures in Hawaii.

Oxide, sulphate and carbonate forms of Mg are reported to have 70% availability to horses although the country of origin of the oxides may affect their availability (Frape, 2010). Supplementary sources of Mg are more available to growing foals than natural sources (NRC, 2007). True digestibility of Mg in horses was reported to be 62 - 76% and was independent of NaCl intake (Schryver *et al.*, 1987). Urinary and faecal losses of Mg of 4.6 mg/kg BW are experienced by horses and a minimum maintenance dose of 10 mg/kg BW is recommended (Frape, 2010). Mg inclusions > 1 % of intake will depress feed intake in horses (Vervuert, 2008).

Human and rat studies show high Mg content in the diet modifies the response to exercise stress with the production of lower levels of stress hormones. High Mg intakes may therefore benefit horses in work (Vervuert, 2008) although feeding high doses of Mg to horses in low intensity exercise showed no benefit (Meyer *et al.*, 1992). Mg levels in the blood were lowered in exercising horses fed high levels of Ca and P (Vervuert *et al.*, 2006) while normal P intake did not affect Mg uptake (Frape, 2010).

2.4.4 Trace minerals

There is a lack of knowledge about exercise requirements for trace minerals and generally recommendations are based on maintenance and growth states, extrapolation from other species and the absence of clinical signs of deficiency (Vervuert, 2008). The potential exists for exercise to increase the need for trace minerals in the diet to optimise biological processes and replace sweat losses but this still needs to be confirmed by further research (Vervuert, 2008). NRC (2007) give recommendations and not requirements for trace minerals as further work is needed to evaluate accurate recommendations in all classes of horses for trace minerals. KER requirements for trace minerals are currently higher than current NRC recommendations in various classes of horses (Pagan, 2009).

2.4.4.1 Copper

Cu-containing ferroxidase enzymes facilitate the conversion of ferrous (Fe^{3+}) Fe to the usable ferric (Fe^{2+}) form in the body which can then be mobilised and used where necessary. Without Cu, Fe^{3+} stores can build up in the liver and anaemia can develop due to the deficiency of Cu rather than Fe and is a more likely cause of anaemia in horses where dietary Fe levels are high (Kellon, 2008a). Excess Cu is excreted via bile into the faeces and is linearly proportionate to the dietary intake (Cymbaluk *et al.*, 1990). Supplementary sources of Cu used for horse feeds are copper sulphate, copper carbonate, copper proteinate, copper chloride and copper chelates, with true digestibility ranging from 24 – 54% (NRC, 2007).

Endogenous losses of Cu have been estimated between 2.9 mg/100 kg BW in sedentary horses, 3.8 mg/ 100 kg in exercising horses (Hudson *et al.*, 2001b) and 3.5 mg/100 kg BW in ponies (Cymbaluk *et al.*, 1990). Horses have a reasonable tolerance for Cu as shown by Smith *et al.* (1975) who fed Shetland ponies 791 mg/kg of feed for 6 months. The ponies had raised Cu liver content but this did not have an adverse effect on the ponies in terms of liver damage or fertility. Dietary content of 15 – 20 mg/kg DM are sufficient to give normal hepatic Cu levels (Frape, 2010).

Foals require 25 – 30 mg/kg DM Cu to reduce the risk of cartilage erosion while growing horses need 15 – 20 mg/kg of dry feed (Frape, 2010). Cu supplementation of the pregnant mare provides a source of hepatic Cu for growing foals until they are able to eat on their own and supplementation of mares reduced the physitis indices and the articular cartilage lesions in foals (Pearce *et al.*, 1998). By increasing Cu intake from 6 - 30 mg/kg DM of feed in grazing mares, the foetal Cu stores increased by 60% (Frape, 2010). NRC (2007) suggests the Cu requirement for horses at maintenance to be 0.2 mg/kg BW while those for horses in heavy exercise are set at 0.25 mg Cu/kg BW (Table 2.10).

Table 2. 10 Trace element requirements of growing, working and breeding horses based upon NRC (2007) recommendations (mg/kg BW per day, except for ADG)

	CU	ZN	MN	FE
MAINTENANCE FOALS	0.22	0.82	0.82	0.8
GROWTH (MG/KG ADG/D)	3.33	30.0	30	80.0
MAINTENANCE ADULTS	0.2	0.8	0.8	0.8
MAINTENANCE LIGHT WORK	0.2	0.8	0.8	0.8
MAINTENANCE MODERATE WORK	0.23	0.9	0.9	0.9
MAINTENANCE HEAVY WORK	0.25	1.0	1.0	1.0
GESTATION 1 – 8 MONTHS	0.2	0.8	0.8	0.8
GESTATION 8-11	0.25	0.8	0.8	1.0
LACTATION	0.25	1.0	1.0	1.25
STALLION	0.2	0.8	0.8	0.8

2.4.4.2 Iron

Horse feeds are naturally high in Fe and a deficiency is very unlikely in horses under normal conditions (Jackson, 2000a; NRC, 2007; Kellon, 2008a; Frape, 2010). Fe forms part of the haem molecule in red blood cells and is often supplemented in horses in the mistaken belief that haemoglobin and packed cell volume are improved while very little evidence shows this happens in the horse (Jackson, 2000b; Pagan, 2001; Frape, 2010). Recommendations for dietary Fe are set at 40 mg/kg DM for horses at maintenance with work, growth and pregnancy at 50 mg/kg DM although NRC (2007) state these may be too high.

The body has no known Fe excretion system and excess Fe builds up in the liver and spleen where it is bound to storage proteins and kept out of circulation to prevent oxidative damage occurring and if the storage mechanisms in the body are exceeded, liver failure and necrosis can occur (Kellon, 2008a; Vervuert, 2008; Frape, 2010). Fe toxicity depends on varying factors such as disease state, anti-oxidant levels and Vit E and Se status. Adult Thoroughbreds showed signs of Fe toxicity with the addition of 300 mg Fe^{3+} to the feed daily and new-born foals are particularly susceptible to Fe toxicity and can develop icterus, diarrhoea, dehydration and coma and death within 5 days of being given supplemental Fe (Frape, 2010).

2.4.4.3 Manganese

NRC (2007) Mn requirements are 40 mg/kg DM for horses at maintenance and 50 mg/kg DM for horses in growth, work and pregnancy (NRC, 2007) with 36 mg/kg DM being shown to be insufficient for yearling Thoroughbreds by Sobota *et al.* (2001; in Frape, 2010) who recommended intake of a minimum 40 mg/kg for growth. Hudson *et al.* (2001) showed true digestibility dropped with exercise although the drop was not significant. It is apparent that there are exercise related shifts in Mn availability. Due to wide ranges of apparent Mn availability from 28 – 58%, it is impossible to set Mn requirements accurately with the current knowledge (Frape, 2010). Jackson (2000) recommend an intake of 50 mg/kg DM per day for horses in moderate to heavy work.

Carbohydrate and lipid metabolism, glucosamine and chondroitin synthesis in cartilage formation, bone matrix formation and anti-oxidation reactions relying on superoxide-dismutase all depend on the presence of Mn (Frape, 2010). Uptake of Mn is inhibited by high Ca levels in the diet and growing horses on lucerne fodder may be prone to flexural deformities, which are improved by Mn supplementation (Frape, 2010). Supplementary sources of Mn used for horses include oxides, sulphates and organic chelates or proteinates.

2.4.4.4 Zinc

Zn is a component of over 100 enzymes and is a co-factor for a further 200 and is critical to the correct functioning of many body systems from immunity, hoof structure, bone formation, anti-oxidant systems, reproduction and skin integrity (NRC, 2007; Frape, 2010). Zn absorption depends on the Zn status of the animal and is reported at 5 – 15% in horses. Conflicting reports exist as to which form of Zn is most available to horses of sulphate, oxide and chelated sources in a number of studies. Cost must be taken into consideration when deciding on a supplementary source for horses (NRC, 2007).

NRC (2007) have kept the Zn recommendation at 40 mg/kg of DM in the diet although field work by Knight *et al.* (1985) in NRC (2007) suggests that the optimal intake is much higher at 90 mg/kg DM. Endogenous losses of 0.1 mg/kg BW equate to 65 – 70 mg Zn/d for a 524 kg horse. Dietary Zn availability is considered to be 20.8%, giving a requirement of 236 mg/500 kg horse, this prompted the NRC (2007) to leave the recommendations at 1989 levels of 40 mg/kg DM (NRC, 2007). Exercise increased the need for Zn from 274 mg/day to 461 mg / day in Thoroughbred geldings as shown by Hudson *et al.* (2001). This agrees the work requirements set by the NRC (2007).

2.4.4.5 Selenium

Se is a potent anti-oxidant which, together with vitamin E, helps protect cells from oxidative damage and is found in the glutathione peroxidase enzyme (Montgomery *et al.*, 2011) which is the only selenoprotein that has been researched in horses (Kellon, 2008a). Se also helps to maintain membrane integrity and is involved in growth, reproduction and immune responses (Pagan, 2001). Young animals which are deficient in Se may suffer from white muscle disease while adult animals tend to have sub-clinical issues related to the role of Se in anti-oxidant, thyroid and immune systems (Montgomery *et al.*, 2011). Selenoproteins are important anti-oxidants and are critical to performance horses where they reduce oxidative damage from free radicals produced in intense exercise raising the requirement for Se in intensely worked horses (Pagan, 2001). Broodmares supplemented with Se showed less placental retention times (Frape, 2010). Adequate Se provision therefore needs to be ensured in hard working and breeding horses. Se toxicity is reported at an intake of 2 mg/kg of diet and the NRC (2007) recommends intakes of 0.1 mg/kg of diet. Research has shown an increase in immune response at intakes of 3 mg per day compared to mares fed 1 mg per day (Pagan, 2001; NRC, 2007). Testing of Se in feed and fodders is costly and the testing of whole blood Se levels is reliable in horses (Kellon, 2008a).

Se can be provided in the form of sodium selenite or sodium selenate and as organic selenium yeasts. Frape (2010) reports the organic and inorganic forms may be metabolised differently in the body and the organic yeast form may be less prone to a reduction in absorption from competition with other inorganic minerals. Plants growing on alkali soils may accumulate Se and incidences of toxicity may occur when horse eat these plants (NRC, 2007).

2.5 Two cases of therapeutic nutrition

Special note must be made of the therapeutic use of mineral intake under the special cases of horse health status. Young, growing horses in work and horses with Equine Metabolic

Syndrome (EMS) require stricter application of mineral provision in their ration and are less tolerant of deviations from requirements, due to the increased demands being placed on them.

2.5.1 Growth in training

Horses complete their growth between 5 – 6 years of age (Bennet, 2008). It is acceptable in the Thoroughbred racing industry to race horses as 2 year olds (Ellis and Saastamoinen, 2008). In this time, special attention needs to be made to supply mineral and protein needs of these horses to ensure strong skeletal growth and to minimise injuries and prolong the performance career of the horse. Muscoskeletal injuries are a major source of lost training days and wastage in racehorses due to micro-injuries which accumulate faster than repair and need days of rest to accelerate healing (Martig *et al.*, 2014). Bone remodelling accommodates the increased load due to speed work experienced by horses in training and bone is most adaptable before maturity so horses worked as two year olds were found to have longer racing careers then horses started at a greater age (Martig *et al.*, 2014). Modelling of bone injuries and repairs is needed to allow trainers to maintain the strain within safe levels (Martig *et al.*, 2014).

Ellis (2008) found fodder inclusions of at least 1.2% of BW are needed for young growing horses in training. Energy requirements for light training during growth may be 20 – 25% higher than maintenance levels (Ellis and Saastamoinen, 2008) while Martin-Rosset (2008) gives maintenance DE requirements of 3 - 4 year old horses as 8% higher than mature horses. Training before skeletal maturity may predispose horses to injuries and nutrition and ability of the skeletal system to adapt to exercise are critical (Ellis and Saastamoinen, 2008). Extra protein is needed to support work and growth requirements of horses in early training (Martin-Rosset, 2008).

The NRC (2007) sets out minimum nutrient requirements for growth from weanlings to 24 months. Ca and P requirements increase in young racehorses as exercise increases (Stephens *et al.*, 2004; Vervuert, 2008) and a risk exists that dietary Ca content is low when the increased need for Ca occurs due to increased bone metabolism in young racehorses (Ellis and

Saastamoinen, 2008). Feeding Ca, P and Mg at 169%, 132% and 168% higher than NRC (1989) requirements increased bone mineral density in two year old racing Thoroughbreds in training and reduced the amount of bone demineralisation compared to horses with lower intakes of these minerals (Michael *et al.,.* 2001; in Frape 2010). Juvenile quarter horses fed 151 % Ca, 130% P and 159% Mg showed greater bone mineral density than horses fed 136%, 98% and 126% NRC content (Nolan *et al.*, 2001).

Currently no set recommendations or requirements are set for growing horses in training for trace minerals. If extra protein and Ca are supplied in the diet then attention needs to be given to the Cu and Zn content of the diet to ensure the reduction in absorption of Zn is accounted for. Ott and Asquith (1989) found yearlings showed greater bone deposition when diets contained 11 mg/kg Cu, 176 mg/kg Fe, 64 mg/kg Mn and 69 mg/kg Zn compared to those fed lower levels. It would therefore be beneficial to ensure growing horses in training received trace minerals in the diet at higher levels than NRC recommendations and in the correct proportions to optimise bone mineral deposition.

2.5.2 Equine metabolic syndrome

Equine metabolic syndrome (EMS) describes a number of symptoms in horses and ponies including obesity, infertility, changes in ovarian activity, insulin resistance (IR), Cushing's disease and laminitis. Underlying the syndrome is often faulty carbohydrate metabolism with characteristic glucose and insulin responses (Merck, 2015).

Management of the disease is primarily dietary and special attention needs to be given to sugar or NSC (non-structural carbohydrate) intake and as more is understood about the disease and its causes, certain mineral changes to the diet do prove helpful. Fodder properties and the rate of intake need to be considered along with DE when choosing feed for restricted diets (Ellis *et al.*, 2015) and hay provided to EMS horses needs to be tested for NSC. Generally grazing is severely restricted or not allowed in EMS cases and hay provided needs to be below 10% NSC (Merck, 2015).

Nielsen *et al.* (2012) showed a potential link between insulin resistance and Fe overload in horses, while in humans the link between the two has been shown (Bozzini *et al.*, 2005). Kellon (2006) conducted a field study on IR horses and found significant differences in ferritin and transferrin saturation indices in IR horses on unbalanced diets compared to control horses and IR horses on balanced diets with controlled trace mineral ratios. Serum Fe levels were significantly higher in IR horses on unbalanced diets compared to control horses which further confirms Nielsen's findings of the link between Fe excess and IR.

Mg deficiency is linked to increased risk of developing Type II diabetes in human adults and children and is an important cofactor in carbohydrate metabolism (Huerta *et al.*, 2005). Horses with marginal Mg status may be more prone to IR and inflammatory reactions involved in laminitis via similar mechanisms. Stewart (2011) cautions against assumptions carried from human to horse nutrition but does report anecdotal evidence from veterinarians reporting improvements in obesity and laminitic episodes with Mg supplementation.

2.6 Ration evaluation

Kohnke (1998) outlines a useful practical guide for ration evaluation. In a more empirical fashion, Emmans and Kyriazakis (2001) use modelling theory for feed intake determination in animal species. Modelling of intakes in equine nutrition is limited in the literature. What is clear is that a more pragmatic approach to the appraisal of equine diets is necessary.

Chemical analysis can be used as a baseline for the assessment of the nutritive value of a feed which would also depend on the digestibility of the raw ingredients used in the ration. The type and levels of performance of a horse will also determine the nutritive value of a feed and there is a need to be able to predict nutrient availability from feeds (Young, 2011).

Blood analysis for minerals such as P, Mg, Cu and Zn is more likely to give indications of dietary intake compared to those for Ca, and Fe which do not provide accurate information on status (NRC, 2007) although Frape (2010) reports serum Zn as inaccurate in determining Zn status.

Ferritin and transferrin saturation levels along with serum Fe levels can be used to determine body Fe stores via the blood (Smith *et al.*, 1984; Kellon, 2008a). Dangers of blood tests exist when results are taken as absolute truths instead of as a dynamic situation at a specific point in time (Spångfors, 2000). Hair and hoof samples are inaccurate as indicators of mineral status as they may be affected by hair colour and other non-dietary factors (NRC, 2007). Diet evaluation is therefore used as a tool to evaluate most mineral intakes and sufficiency in the diet (NRC, 2007).

Kronfeld (2001) outlines a practical guide to diet evaluation (Figure 2.3), using the formulation of nutrient goals with a range of nutritional variables, rather than a fixed requirement as currently occurs with horse rations. The ration is then evaluated by means of a sensitivity analysis to uncover weaknesses in formulations.

There is no indication that SA horse feed companies have adopted any pragmatic approach to horse feed formulations. Ration evaluation needs to start with requirements and feed intake, before the concentration of nutrients in the ration can be determined. Physiologically, horses, as hindgut fermenters, have the capacity to increase intake but whether it is always necessary has not been pursued. In fact there is no indication in the literature that states horses need to eat to capacity.

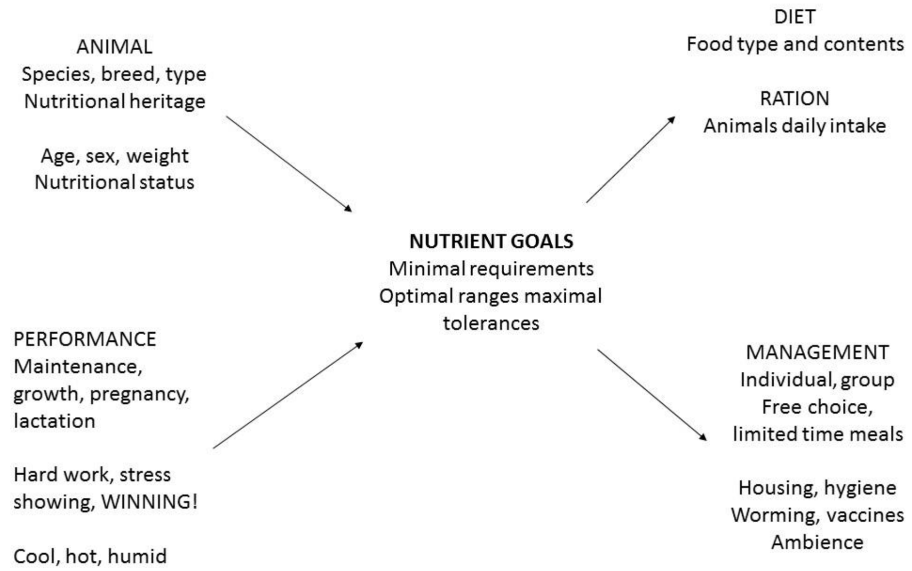


Figure 2. 3 An orientation of nutritional practice focuses on setting nutritional goals for energy and nutrients in keeping with an animal's nature, desired performance and state of health. The goals are optimal ranges which are usually specified as lower, middle and upper values (Kronfeld, 2001).

2.6.1 Determining intake

In order to be able to accurately evaluate a ration, intake of feed and fodder needs to be determined. It is generally accepted that horses will consume between 2 - 2.5% of their body weight per day in dry matter of feed and fodder under normal conditions and up to 3% if needed (NRC, 2007) (Table 2.11). Although the application of the 2 – 2.5% BW DMI is shown to occur in racing Standardbreds and Thoroughbreds, Owens (2005) found sport horses in Australia consume between 1.04 and 2.55% of BW with a roughage component of 40 – 50% of intake. It is clear that intake in horses is variable and more research needs to be undertaken in modelling of feed intake, in particular the effect of nutrient density of concentrate feed needs to be investigated. When applying the principle of first limiting nutrients, horses will eat to fulfil energy and protein requirements (Young, 2011). Using this premise, horses may reduce feed intake when rations supply all the nutrients a horse requires, altering the DMI.

Likewise DMI may increase when supply of essential nutrients is limited often resulting in "grass belly" when horses eat large amount of low quality fodder.

Table 2. 11 Expected feed consumption by horses as given by Pagan (2000)

	% of body weight		% of diet	
Horse	**Fodder**	**Concentrate**	**Fodder**	**Concentrate**
Maintenance	1.0 - 2.0	0 - 0.1	50 - 100	0 - 50
Pregnant Mare	1.0 - 2.0	0.3 - 1.0	50 - 85	15 - 50
Lactating Mare (early)	1.0 - 2.5	0.5 - 2.0	33 - 85	15 - 66
Lactating Mare (late)	1.0 - 2.0	0.5 - 1.5	40 - 80	20 - 60
Weanling	0.5 - 1.8	1.0 - 3.0	30 - 65	35 - 70
Yearling	1.0 - 2.0	0.5 - 2.0	33 - 80	20 - 66
Performance Horse	1.0 - 2.0	0.5 - 2.0	33 - 80	20 - 66

Other factors to consider in the intake assessment are accurate body weight determination, body condition score, physiological state and performance status (Becvarova *et al.*, 2009) as the NRC (2007) now give recommendations on a per kg BW basis for different life stage or work category of the horse. These factors can all be used to assess the dietary status of a horse and to allow owners or managers to reassess dietary requirements as needed.

Little is known about intake control in horses but palatability, especially smell and taste, and nutritional content of a feed will influence intake although large individual variations occur between individual horses (Julliand *et al.*, 2008). Feed intake is regulated in the short term by available glucose and acetate levels and hormonally by leptin and ghrelin production, while long-term ingestion is controlled by the nutritional requirements of the horse which in turn is controlled physiologically by work or activity levels and thermo-regulation requirements (Julliand *et al.*, 2008).

2.6.2 Prediction of feed intake

Studies on voluntary dry matter intake (VDMI) of fodder in horses yield varying results while little information on pasture DMI is available (NRC, 2007). Horses prefer cool season grasses to warm season grasses and VDMI of cool season grasses is generally higher than warm season ones (McCown *et al.*, 2012). Attempts to link DMI to chemical characteristics of fodder have failed to show consistent results (Dulphy *et al.*, 1997; NRC, 2007). Fodder DMI of horses has been shown to be in the region of 16 - 20 g/kg BW or 1.5 - 3.1% of BW (NRC, 2007; Edouard *et al.*, 2009). Lucerne hay with a DMI of 2.4% BW shows slightly higher intake values than those of grass hays at 2% BW. Individual horses show a definite change in DMI with the majority of horses increasing DMI when fodder quality decreases while, other horses show decreased or unchanged intakes with declining CP and increasing NDF values in grass hays and fresh fodder (Edouard *et al.*, 2008). Restricting grazing time on pastures increased intake rate and yielded the same DE intake in pasture plus hay or on *ad libitum* pasture (Glunk *et al.*, 2013).

Unlike with pastures, the DMI of hay and feeds is easier to calculate, due to the ease of providing set quantities of each to the horse and calculating intake. A 2001 review of hay intake by Lawrence *et al.* (2001) found NDF to be correlated to VDMI while CP was not. They also found horses preferred hays with an NDF < 65% and reduced their intake if the NDF was higher than this. McCowen (2012) also found horses prefer the lower NDF and ADF of early cut versus late cut teff. The digestion and utilisation of nutrients in horses is dependent on the rate of passage of feed through the intestines (Van Weyenberg *et al.*, 2006) and modern feeding practices providing one or two large meals a day and limited fodder provision increase the risk of digestive issues (Julliand *et al.*, 2008).

As reported by the NRC (2007) DMI of horses seems to be more commonly 2 - 2.5% of BW. Intakes are worked out to provide NRC requirements for DE and CP levels and the mineral intakes are calculated from the amount of feed and fodder the horse would consume to meet these targets.

2.6.3 Fodder to concentrate ratio

Even though many horse owners recognise the need for high fodder inclusion in horse diets, this is not always provided to horses. Horses are still fed high rates of concentrates as there seems to be a fear of not supplying sufficient nutrients and having impaired performance and appearance from a gut full of grass by many trainers and owners (Longland, 2012). Horses need to fulfil ingestive behaviour for a minimum of 8 - 9 hours per day with an average of 12.5 ±2.5 hrs per day irrespective of diet and if this need is supressed they began to ingest bedding, shavings and turn to coprophagia to fill this time. The reduction in chew time also impacts negatively on the GIT with reduced passage rate and increased ulcer and colic rate and increased likelihood of stereotypical behaviour (Ellis *et al.*, 2010; Ellis *et al.*, 2015). Methods of increasing ingestion time are to add chaff to the feed, provision of multiple fodder types and the use of small nuts in concentrated feed (Ellis *et al.*, 2015). The balanced provision of fibre and starch in horse rations, with the addition of a probiotic, limits changes in intestinal microbial colonies (Young, 2011).

Meals containing high levels of fermentable carbohydrates will lead to slower stomach emptying and better digestive health of the horse (Julliand *et al.*, 2008). The caecum and colon are major sites of NDF digestion and NDF digestion is increased when high grain diets are fed compared to fodder diets (Hintz *et al.*, 1971), indicating some improvement in hindgut microbial fermentation in the presence of carbohydrates.

Many SA feed companies recommend concentrate feeding rates of 4 kg for idle horses up to 8 and 10 kg for working horses which provides hard feed levels in excess of recommendations if the minimum fodder intake of 1 - 1.5% of total dietary intake is used. Total intake is assumed to be in the region of 2 - 2.5% for a 500 kg horse and would equate to 5 – 7.5 kg of fodder. Hackland (2007) found Thoroughbred racehorses were fed fodder to concentrate (F:C) ratios of 28:72 and 22:78 which is far in excess of the 40:60 recommended by the NRC (2007) and Kohnke (1998) for racehorses in training. Young (2011) categorised F:C for varying classes of horses as 80:20 - idle, 50:50 - working horses and race as 20:80 which will be used in this thesis when calculating diets for various categories of horses.

2.6.4 Nutrient intakes

Intakes of all raw materials and their nutrient content will determine nutrient intake. The digestible energy of feeds and fodder for horses can be calculated using the following formulae derived by work done in 1981 by Fonnesbeck (NRC, 2007; Longland, 2012) for fodder

$$DE\ (MJ/kg\ DM) = 4.184 \times (4.22 - (0.11 \times ADF\ \%) + (0.0332 \times CP\ \%) + (0.00112 \times (ADF\ \%)^2)$$

Feeds and protein supplement energy calculations are given by the NRC (2007) as

$$DE\ (MJ/kg\ DM) = 4.184 \times (4.07 - (0.055 \times ADF\ \%))$$

CP intake is a function of intake and concentration of CP in the raw materials. As the two major nutrients that for the most part determine intakes as a whole (First limiting nutrient theory), DE and CP usually dictate intake levels of all fibre and minerals. Mineral form and the presence of antagonistic nutrients (phytates, oxalates) as well as balance between minerals known to interact with one another, need to be carefully considered in the provision of minerals.

Where intake should usually be to satisfy the requirement for specific species, over provision of palatable, nutrient-dense feed, exacerbates existing nutritional imbalance. This usually happens where there is a shortcoming in the development of the rational feeding model.

Chapter 3 *In Vitro* Fodder Analyses

3.1 Introduction

Horses in SA are kept under varying management regimes and are fed varying proportions of fodder and concentrate in their ration depending on the way they are kept - from horses on total fodder diets who generally graze what is available to them with little additional feed through to racing Thoroughbred's receiving 80 % of their ration in concentrate feed with very little hay fed as roughage (Table 3.1) (Hackland, 2007). The large numbers of horses being kept in more urban environments has increased the numbers of horses eating hay year round as grazing in these areas is very scarce to non-existent.

Table 3. 1 Dry matter intake and percentage contribution of fodder and concentrate to the ration in various groups of horses (Sources: Owens, 2005; Hackland, 2007; NRC, 2007; McCowan, 2011; Young, 2011)

Horse	DM intake as % of Body weight	% Fodder	% Concentrate
Idle	2	100	0
Hack	2	80	20
Sport	1 - 2.5	50	50
Race	2.1 - 2.5	20	80
Lactating Broodmare	2.5	50	50
Pregnant Broodmare	2	80	20

Sun dried hay is the predominant source of conserved fodder in SA where haylage and artificially dried fodder are seldom produced for use in the horse industry. Grazing for horses varies and a wide range of vegetation types from low nutrient indigenous veld to highly nutritious cultivated pastures (some of which can be irrigated) are used and many grazing horses are supplemented with hay over winter months when grass growth slows or stops.

As horses are seldom offered a variety of fodder types any nutrient deficiencies or excesses are compounded by the length of time a horse consumes the fodder for and can be exacerbated by the provision of poorly balanced feeds that do not correct nutrient imbalances in the fodder. DE and CP imbalances will result in visual changes occurring in body condition of horses and can be rectified when the horse owner or manager notices these changes whereas mineral imbalances are seldom visually evident and may not be noticed at all until severe deficiencies occur. It is therefore important to understand what minerals a fodder supplies to the horse and to formulate feeds to complement the fodder portion of the diet. Pagan (2000) states "Forage should remain the foundation of a horse's feeding program, regardless of where it is raised or how it is used. Additional grains or protein and mineral supplements should only be used to supply essential nutrients not contained in the forage."

Large variations in nutrient content of a fodder can be obtained with fertiliser and lime applications in cultivated fodder while naturally occurring veld grasses tend to grow on poor soils and have lower mineral content and CP content (Longland, 2012). Species, maturity, area and soil type, as well as production practices experienced in the growing season all impact the nutritional properties of plants. Plant mineral uptake is affected by soil pH and practices such as liming, which alter soil pH, can have an impact on plant mineral content (Figure 3.1). Fodder growing in the acidic soils will contain higher content of Fe and Mn and less Cu, Zn, Ca, Mg and P. The wetter, eastern areas of SA tend to have acidic soils (pH (KCl) 4 – 5) with pockets of alkali soils in the lower lying bushveld areas where Mn deficiencies are often experienced (Farina, 2014).

Suttle (2010) shows P content in plants can be elevated by the application of phosphate fertiliser in excess of plant needs and can supply grazing animals with extra P while low soil P content will reduce the plant, and therefore the animal intake of P. In fodder containing oxalates, such as kikuyu, Ca and possibly Mg are unavailable to horses and the excess P often found in these pastures puts horses at risk of developing nutritional secondary hyperparathyroidism from Ca deficiency or inverse Ca:P intake. Nitrogenous fertiliser application will increase the CP, amino acid content and growth of fodder (Blumenthal *et al.*, 2008; Longland, 2012) while the addition of sulphate fertilises will increase the sulphur containing amino acids such as methionine and cysteine in plants (Mortensen *et al.*, 1992). Zn and Cu applied in the fertiliser can increase the trace mineral content of plants and would improve their intake in animals eating these plants (Suttle, 2010).

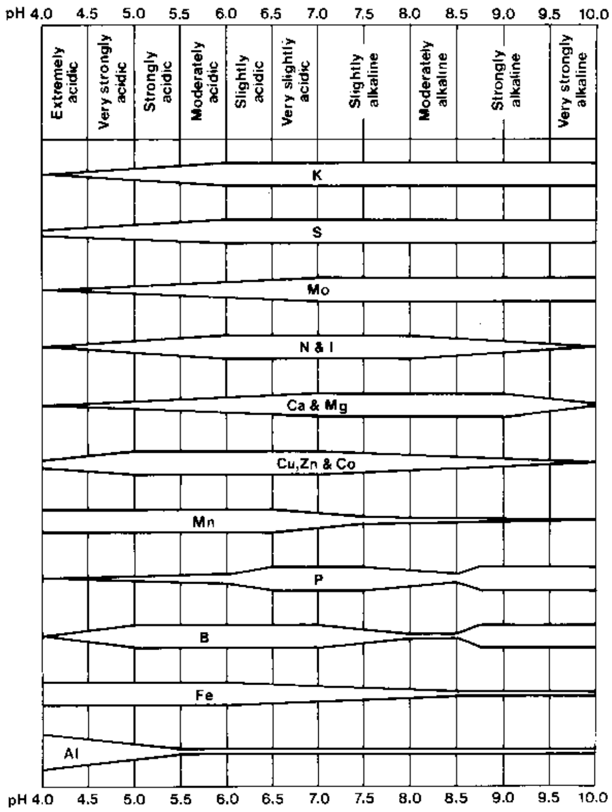


Figure 3. 1 General trend of the influence of reaction (pH) on the availability of plant nutrients (widest part of the bar indicates maximum availability) - Organic soils (after Lucas and Davis, 1961) (FAO, 2015)

In SA fodder types tend to be area specific, with water availability determining the type of fodder grown in the different regions. The drier western areas of the country favour the production of oat-hay and lucerne while the wetter eastern areas of the country favour the production of grass hays such as eragrostis (*Eragrostis curvula)*, teff (*Eragrostis teff)* and veld hay with smaller amounts of lucerne (*Medicago sativa)* and oat-hay (*Avena sativa)* being cultivated and used for horses. Kikuyu (*Pennisetum clandestinum)* based pastures are common throughout SA and horses graze these during the wetter months and receive supplementary hay in the dry season when growth of the kikuyu slows.

As consumers, horse owners have very little input into growing conditions of fodder and buy what is available to them. Additionally no nutrient specification sheets for mineral content in fodder exist for feed companies to formulate complementary feeds from. It would be beneficial to understand the various fodder types and their potential nutrient properties to be able to match feed and fodder types to the horse's work and physiological status. A fodder analysis would give a solid foundation of nutrient content and would be a valuable tool in defining criteria necessary in feed and fodder selection to ensure optimal mineral nutrition.

In an attempt to assess the daily mineral intakes of horses, this study was undertaken to analyse fodder types used in the horse industry in SA. Chemical analysis of various types of fodder, used to feed horses, was carried out by a commercial feed laboratory and used to ascertain whether the varying fodder types have distinct mineral profiles to allow for a better understanding of the contribution these make to the mineral intake of horses.

The selection of classifying variables used in this study define characteristics of the fodder which are easily determined and which have been shown to affect daily intakes for horses or are legal requirements in the manufacture of SA feeds under Act 36 (1947). DE, CP, and fibre fractions (NDF and ADF) have been shown to influence a horse's intake of fodder. Ash content give an indication of the total mineral content of a fodder. Fat is included as a potential variable due to its contribution to DE of a fodder, while Ca:P content was included as a defining factor in mineral uptake from oxalate-containing grasses and are a legally required parameter in horse feed production.

It is hypothesised that classes of fodder can be generated based on species, geographical area, cultivation and preservation practices and the subsequent nutrient content. It is important to define whether mineral content is expected to vary over grass classes of differing nutrient content as well.

Horse owners and feed manufacturers need to be aware of the contribution fodder types make to the daily mineral intake of horses. Feeds should ideally offer some cognisance of the mineral intake expected from fodder sources to provide optimal daily nutrient intake.

VIMI packs are formulated with no cognisance of the contribution of minerals from the fodder portion of the ration. Additionally no such current fodder mineral database for horses in SA exists.

This study intended to determine what groups of fodder can be generated and what generic mineral contents these classes of fodder confer for subsequent feed and ration formulation.

3.2 Materials and Methods

3.2.1 Chemical analysis

Between April 2011 and May 2015, 99 fodder samples were collected from around South Africa (including Swaziland) from horse owners and stable yards.

Samples of grazing were taken in the field - horse owners were asked to observe the grazing habits of the horses and then select proportionate amounts of the species of grasses, preferred by horses, for sampling. Multiple samples were cut to the height horses grazed them to, pooled for homogeneity and dried, if necessary, then bagged and labelled prior to being submitted to the laboratory for testing.

Hay samples were collected from various bales of the same batch of hay and were obtained from the inside of the bales to eliminate oxidative changes occurring on the outer hay layers.

Samples were then pooled to ensure homogeneity of the sample before being bagged, labelled and sent to the laboratory for testing.

Fodder samples were submitted to the Cedara Feed Laboratory in KwaZulu-Natal, South Africa for analysis. Samples were weighed and dried then milled through a 1 mm sieve in preparation for analysis. The chemical characteristics were determined as follows: DM % was obtained by drying of samples overnight in an oven at 100 °C. Fat content was determined by the ether extraction method (AOAC 920.39), Ash content was determined (AOAC 942.05), ADF and NDF fractions were determined using the van Soest method on a digestion block (Van Soest *et al.*, 1991) and values were inclusive of residual ash. CP was calculated using the conversion factor of 6.25 (AOAC, 1990) after N was determined using the Dumas method (AOAC 698.06). Ca, P, Mg, Cu, Fe, Mn and Zn content was determined using the Hunter method (Hunter, 1984) and after digestion the minerals were read on an induction coupled plasma - atomic emission spectrometer (Varian ICP-OES Model MPX).

All figures were corrected to enable reporting on a 100% dry matter basis and results of the analyses were tabulated using Excel (MSOffice 2013). Ca content in kikuyu - an oxalate producing species, were reduced to 20% of analysed values to account for the reduction in availability experienced by horses grazing oxalate producing fodder (Stewart *et al.*, 2010). Ca:P and Fe:Cu ratios were determined and DE values were calculated using the following equation of Fonnesbeck *(NRC, 2007; Longland, 2012)*:

$$DE\ (MJ/kg\ DM) = 4.184 \times (4.22 - (0.11 \times ADF\%) + (0.0332 \times CP\%) + (0.00112 \times (ADF\%)^2)$$

Hemicellulose content was calculated using the following equation given by Pagan (2000):

$$Hemicellulose\ \% = NDF\% - ADF\%$$

3.2.2 Statistical analysis

Genstat v14 (2014) was used for statistical analysis. In order to create distinct fodder groups, classifying variables (Ash, ADF, CP, Ca:P, DE, Fat, hemicellulose and NDF) were identified and a correlation matrix of classifying variables was constructed to reduce multi-collinearity between variables. A PCA was carried out using a variance-covariance matrix to calculate PC scores to maximise nearest neighbour linkages. PCA Biplots were generated to elucidate the significance of the relevant classifying variables. Biplots were predictive with a Convex Hull showing distinctions between fodder groups and the mean nutritional properties of the groups were then calculated to use as a basis for comparison in an unbalanced ANOVA in Genstat v14 (2014).

The first PCA was used to discredit certain obvious outliers, before the balance of the more similar fodder types were segregated on the basis of their proximate and chemical characteristics. The PCA fodder groups were used to assign a treatment structure in Excel (MSOffice 2013). Tables were constructed for all fodder groups and an unbalanced ANOVA in Genstat v14 (2014) was used to calculate means for the classifying variables as well as the corresponding mineral content of all fodder types.

3.3 Results

The chemical analysis of the fodder samples were tabulated in Excel (MSOffice, 2013). The calculation of DE, Ca:P, Fe:Cu and hemicellulose was added. A correlation matrix showed a high positive correlation between DE, NDF, CP and ADF while ADF and NDF were negatively correlated with CP (Table 3.2). CP content usually drops as the proportion of fibre in fodder sample increases with maturity (Pagan, 2000; Longland, 2012). CP and ADF are used in the calculation of DE (NRC, 2007; Longland, 2012) and should be correlated. Consequently the ADF, Ca:P, CP and NDF were used in the primary PCA.

Table 3. 2 Correlation matrix using nutrient content of 99 fodder used to reduce multi-collinearity between classifying variables to be used in subsequent PCA analysis.

ADF	**-**							
Ash	-0.3618	-						
CP	-0.7540	0.6001	-					
Ca:P	0.3399	0.0349	-0.3205	-				
DE	-0.8308	0.6076	0.9669	-0.2891	-			
Fat	-0.4720	0.1948	0.5067	-0.3543	0.4948	-		
Hemicellulose	-0.0025	-0.5725	-0.3488	-0.3898	-0.3015	0.1309	-	
NDF	0.6769	-0.6669	-0.7685	-0.0563	-0.7858	-0.2240	0.7344	-
NFE	-0.245	0.4080	0.1474	0.4265	0.2057	-0.2607	0.7830	0.7430
	ADF	**Ash**	**CP**	**Ca:P**	**DE**	**Fat**	**Hemicell**	**NDF**

Two PC scores using the classifying variables ADF, Ca:P, CP and NDF, accounted for 94.28% of the variation between samples. Eigen values in the latent vector loadings (Table 3.3) show variation between samples is primarily due to NDF content, with ADF and CP both contributing lesser proportions to PC score 1 (82.73%) while PC score 2 (11.55%) was defined by ADF and NDF with a smaller contribution from CP and Ca:P.

Table 3. 3 Latent vector loading scores using ADF %, CP %, Ca:P and NDF % as classifying variables to distinguish between fodder types, showing NDF, ADF and CP have the largest effect on fodder variation in PC Score 1 and 2 (94.28%) in a PCA using Genstat v14 (2014)

	PC Score 1 (82.73%)	PC Score 2 (11.55%)
ADF	0.45566	0.72159
CP	-0.44788	-0.28763
Ca:P	0.01465	0.19526
NDF	0.76913	-0.59871

A PCA Biplot was generated to show the various groups of fodder (Figure 3.2). The high CP, low ADF and high Ca:P content of lucerne placed it apart from the other fodder as seen by its position in the upper left quadrant of the Biplot. Kikuyu was differentiated from the other fodder primarily on the basis of its high CP content. Its position to the left of the other fodder types but on the same horizontal plane indicates it has similar content of ADF to other groups but the lower NDF content moved it to the left of the main group of fodder. Lucerne differed from kikuyu by having low NDF, high Ca:P ratio and higher ADF values. The bulk of the fodder occupy a space defined by the following ranges

NDF	70 - 80%,
ADF	40 - 50%,
CP	6 - 15%
Ca:P	1 - 3

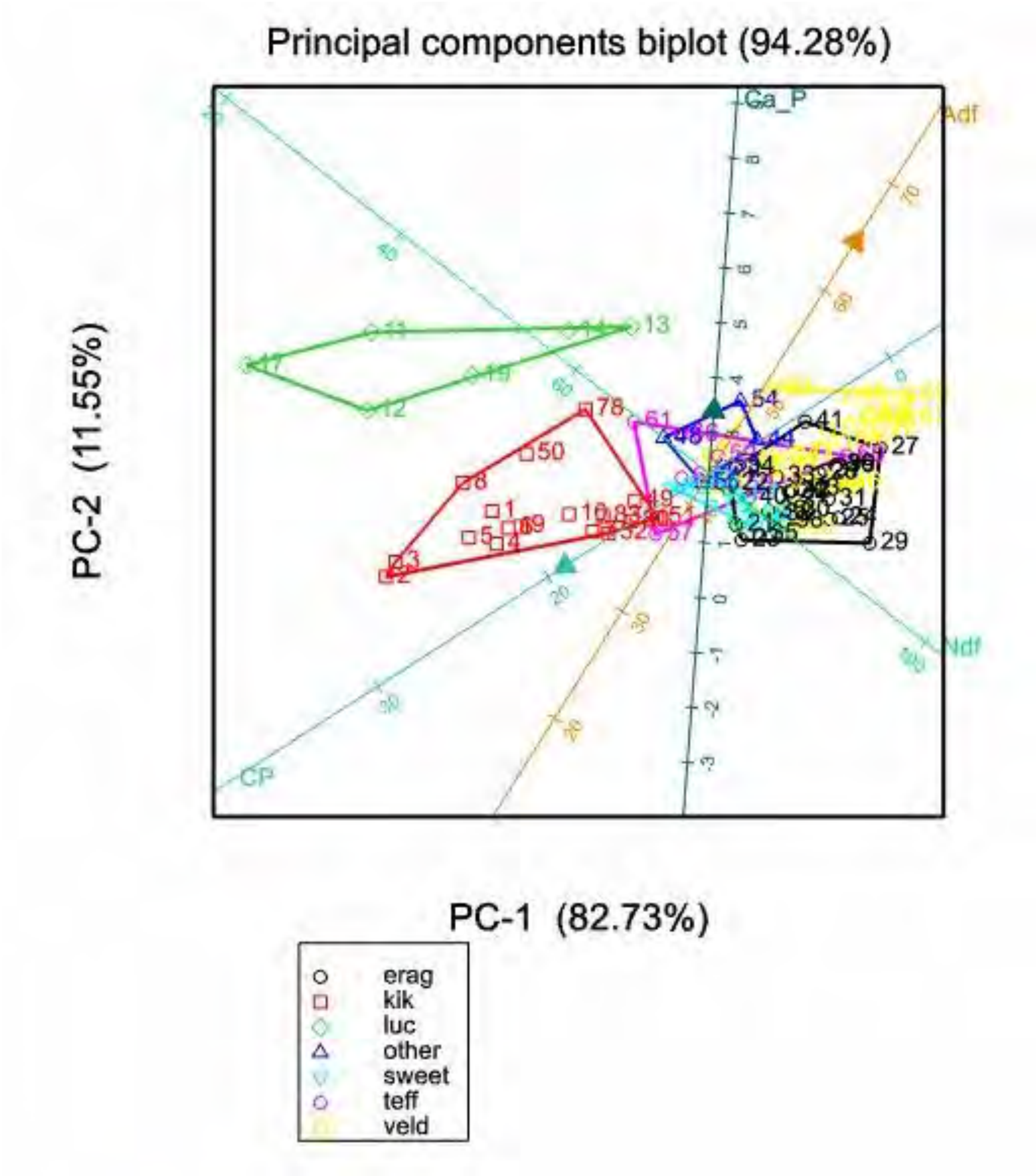


Figure 3. 2 Principle Component Analysis Biplot using ADF%, Ca:P ratio, CP % and NDF % as classifying variables, showing kikuyu and lucerne as distinct groups, separate from all other fodder types

Kikuyu and lucerne samples were removed from the group, leaving 73 fodder samples of eragrostis, mixed hay, sweetveld, teff and veld groups for further analysis. A second PCA was carried out on these fodder samples with the classifying variables CP %, ADF %, NDF % and hemicellulose % accounting for the highest variation of 92.39% between samples. Table 3.4 shows the Eigen values in the latent vector loadings of PC score 1 (47.11%) showed ADF and NDF contributed to most of the variation between samples with less of an influence from CP and hemicellulose while PC 2 score (45.28%) showed a higher loading value from hemicellulose and NDF content with little effect from ADF and CP.

Table 3. 4 Eigen values making up latent vector loadings indicating the contribution each classifying variable (ADF %, CP %, Hemicellulose % and NDF %) makes to PC score 1 and 2 (92.39%).

	PC Score 1 (47.11%)	PC Score 2 (45.28%)
ADF	0.76359	0.15985
CP	-0.30757	0.03624
Hemicellulose	-0.25773	-0.77288
NDF	0.50587	-0.61302

As can be seen from the Biplot (Figure 3.3) the various fodder groups overlap each other with the group positions on the horizontal plane influenced by variations in the ADF and CP content.

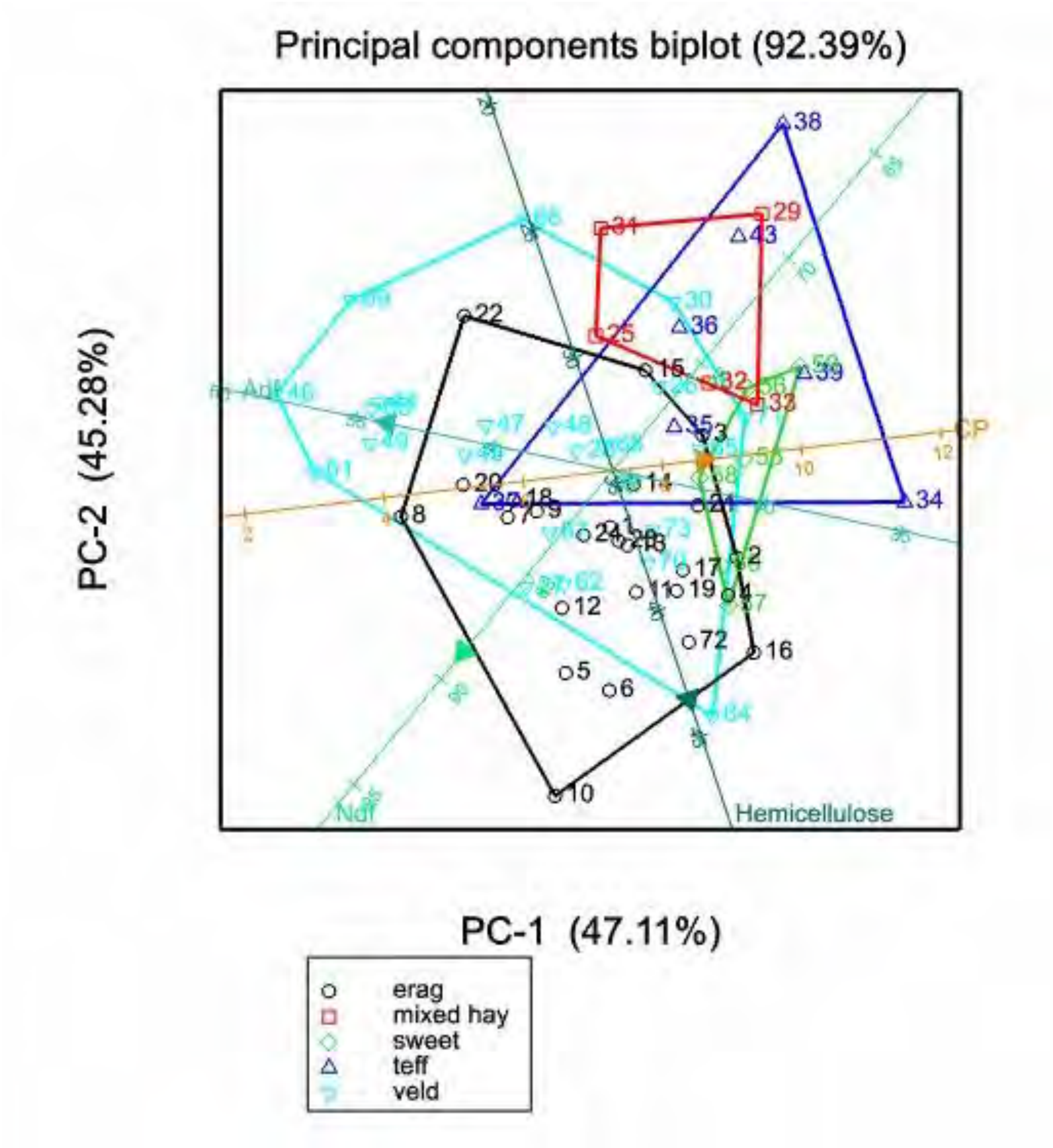


Figure 3. 3 Principle Component Analysis Biplot using ADF%, CP%, hemicellulose % and NDF % as classifying variables, conducted on 73 fodder samples, excluding lucerne and kikuyu samples

Eragrostis, sourveld and teff form large groups indicating wide variations in nutrient content. The position of eragrostis below the mixed hay group and the bulk of the teff group, results from its higher NDF and hemicellulose content and the majority of the eragrostis samples have less CP and higher ADF values than teff, sweetveld and mixed hay. Veld tends to have lower CP than teff, sweetveld and the mixed hay group and has similar CP values to eragrostis. Sweetveld forms a small group to the right of the centre group and has higher CP and lower ADF content than eragrostis and shows a narrow variation in NDF values of between 70 - 75% with hemicellulose values of 40 – 45% which places it in the mid-range for these latter values. Teff hay contains lower fibre (ADF, NDF and hemicellulose) content and more protein than the bulk of eragrostis and veld grasses. Mixed hay occupies a space to the upper right of the groups due to its low hemicellulose, NDF and higher CP content than eragrostis, sweetveld and the majority of veld group.

Within the latter group of fodder samples (excluding kikuyu and lucerne) the major minerals appear to offer some distinction between fodder types as indicated by the combined PC Score (87.63%) (Table 3.5). Ca and P contribute more to the Eigen vector loadings of the PC scores than Mg. The teff and veld group show a wide range of major mineral content and occupy a large area of the Biplot while eragrostis, mixed hay and sweetveld are reasonably compact groups indicating smaller variations between major mineral content compared to teff and veld. Veld has overall lower mineral content than the other fodder types as shown by the bulk of its area being to the left of the main group (Figure 3.4). Sweet veld lies more to the right of the groupings and has higher mineral content than eragrostis, mixed hay and most of the veld group.

Table 3. 5 Eigen vector loading scores making up PC Score 1 and PC Score 2 (87.63%) using Ca %, Mg % and P % as classifying variables in a PCA using Genstat v14 (2014) which accounts for the variation between 73 fodder samples (excluding kikuyu and lucerne)

	PC Score 1 (62.85%)	**PC Score 2 (24.77%)**
Ca	0.62964	-0.74665
Mg	0.43768	0.11264
P	0.64187	0.65562

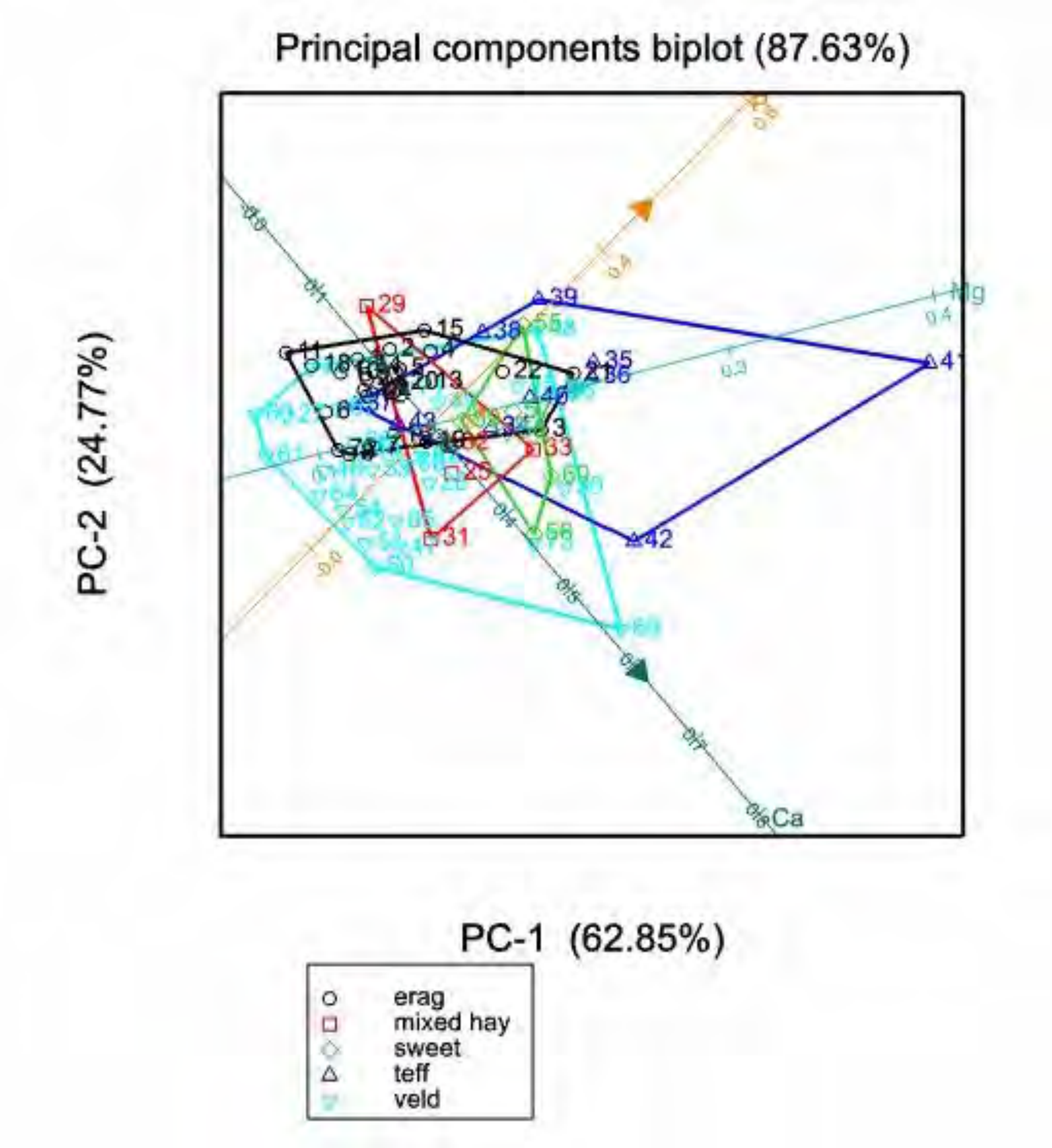


Figure 3. 4 Principle Component Analysis Biplot using Ca %, Mg % and P % as classifying variables conducted on 73 fodder samples, excluding lucerne and kikuyu samples

Using trace mineral content (Fe mg/kg, Fe:Cu, Mn mg/kg) of the 73 fodder samples (excluding kikuyu and lucerne) in a PCA, latent vector scores indicate Fe is responsible for the majority of variation between fodder (Table 3.6). The Biplot shows the majority of samples clustered together with some veld samples extending to the extreme right of the plot due to their very high Fe content (Figure 3.5).

Table 3. 6 Latent vector loading scores using Fe mg/kg, Fe:Cu and Mn mg/kg as classifying variables to distinguish between fodder groups showing Fe and Mn have the largest effect on fodder variation in PC score 1 and 2 (96.68%) in a PCA using Genstat v14 (2014)

	PC Score 1 (92.63%)	PC Score 2 (4.05%)
Fe	0.96345	0.02769
Fe:Cu	0.25807	-0.3627
Mn	0.07185	0.93149

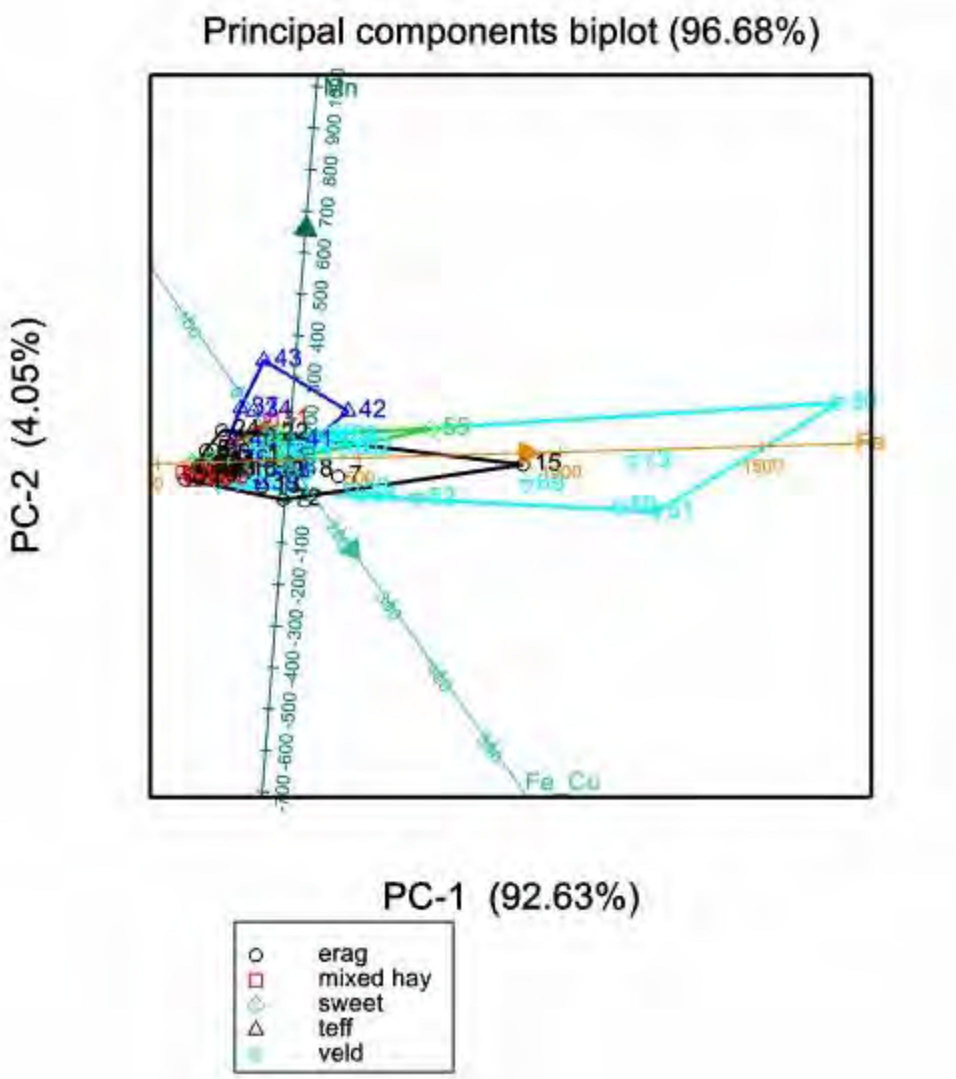


Figure 3. 5 Principal Component Analysis Biplot using Fe mg/kg, Fe:Cu and Mn mg/kg as classifying variables conducted on 73 fodder samples excluding kikuyu and lucerne samples

The group structure elucidated by PCA was used as a treatment structure in an Unbalanced ANOVA Genstat v14 (2014) to obtain the treatment means. The treatment means of each fodder type are the basis for feed formulation assumptions of nutrient intake (fibre, CP, DE and macro and trace mineral intake) in the daily ration of the horse. Significant differences (P < 0.001) in NDF, Ca, Mg and Ca:P ratio occur between lucerne and kikuyu while Mn content differ less significantly between the two (P = 0.004) (Table 3.7 & 3.8).

Table 3. 7 Mean nutrient values of various South African horse fodder types showing fibre, energy, protein and Ca:P content on a 100% DM basis

Fodder	*n*	ADF %	NDF %	Hemi-cellulose	DE MJ/kg	CP %	Ca:P
Eragrostis	25	45.34 ^{ab}	82.59 ^{a}	37.24 ^{a}	7.66 ^{b}	7.72 ^{bc}	1.45 ^{bc}
Kikuyu	17	33.83 ^{c}	65.64 ^{d}	31.81 ^{b}	10.64 ^{a}	20.29 ^{a}	0.60 ^{c}
Lucerne	9	39.32 ^{bc}	49.84 ^{e}	10.52 ^{c}	10.04 ^{a}	18.50 ^{a}	4.83 ^{a}
Mixed hay	5	43.88 ^{ab}	73.70 ^{c}	29.82 ^{b}	7.62 ^{b}	7.22 ^{bc}	2.28 ^{b}
Sweetveld	6	41.45 ^{b}	77.60 ^{b}	36.15 ^{ab}	8.24 ^{b}	10.60 ^{b}	1.58 ^{bc}
Teff	10	42.45 ^{ab}	74.21 ^{c}	31.77 ^{b}	7.96 ^{b}	8.67 ^{bc}	1.36 ^{bc}
Veld	27	48.42 ^{a}	81.28 ^{a}	32.86 ^{ab}	7.32 ^{b}	5.47 ^{c}	3.69 ^{ab}
Overall Mean	99	42.90	75.24	32.34	8.37	10.56	2.26
LSD (max)		6.03	5.64	5.21	0.95	3.98	1.68
SED		3.03	2.84	2.62	0.48	2.00	0.85

$^{a\,b}$ Means in same column bearing different superscripts differ significantly (P<0.001)

Table 3. 8 Mean mineral content of South African horse fodder types reported on 100% DM basis (99 fodder samples)

	n	Ash %	Ca g/kg	Mg g/kg	P g/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg
Lucerne	9	11.14 [a]	10.92 [a]	2.86 [a]	2.48 [ab]	6.78 [abc]	394.00 [ab]	45.11 [b]	24.55 [ab]
Kikuyu	17	10.36 [a]	1.61[1] [c]	1.18 [bc]	3.22 [a]	8.72 [ab]	322.90 [ab]	143.80 [a]	31.54 [ab]
Eragrostis	25	5.04 [c]	2.27 [bc]	1.36 [bc]	1.65 [bc]	4.53 [bc]	225.50 [ab]	72.54 [ab]	20.98 [b]
Mixed hay	5	6.91 [bc]	3.10 [b]	1.36 [bc]	1.74 [bc]	5.04 [bc]	135.50 [b]	66.70 [ab]	20.54 [b]
Sweet veld	6	7.23 [bc]	3.49 [b]	1.63 [bc]	2.29 [ab]	5.00 [bc]	250.80 [ab]	99.71 [ab]	36.88 [ab]
Teff	10	7.98 [b]	3.38 [b]	2.09 [ab]	2.72 [ab]	10.30 [a]	264.80 [ab]	148.90 [a]	43.76 [a]
Veld	27	7.72 [b]	2.89 [bc]	1.54 [bc]	1.10 [bc]	2.92 [bc]	486.40 [a]	113.80 [ab]	20.64 [b]
Mean	99	7.64	0.33	0.16	0.20	5.65	329.65	102.61	26.27
LSD (max)		2.36	0.14	0.08	0.10	5.01	320.40	88.94	17.65
SED		1.19	0.0708	0.04124	0.0496	2.523	161.3	44.78	8.889

[1] Ca calculated @ 20% availability to horses due to oxalate content
[a b] Means in same column bearing different superscripts differ significantly (P<0.001)

3.4 Discussion

As hypothesised, the various groups of fodder can be distinguished from one another based on their proximate analysis. Segregation on the basis of fibre (ADF, hemicellulose and NDF) and CP content enables the formation of groups of similar nutritional content and although five of the groups are not distinctly separate from one another on the basis of their chemical analysis, generalised nutrient content can be obtained for these groups (Table 3.7 & 3.8).

Siciliano (2015) reports DE concentration in pasture to be between 7.5 and 11.32 MJ/kg, and DE content of the fodder in this study fall within this range. The protein and fibre specifications in the analysed fodder fall within similar ranges reported for SA fodder by Bredon *et al.* (1987) and Meissner (1997). However, vast differences do occur within fodder groups depending on growing and production practices in terms of CP and mineral content of fodder. Differing geographical areas could result in varying mineral content of fodder therefore analysis of individual batches of fodder would be beneficial in ration formulation.

Limited data exist for mineral content of local grasses and Bredon *et al.* (1987) show only Ca and P content. International electronic databases such as Dairy One (www.dairyone.com) and Feedipedia (www.feedipedia.org) have limited data on SA species such as *Eragrostis teff* and *Eragrostis curvula* and do not report trace mineral content of these grasses. Besides kikuyu and lucerne, the grasses analysed show no statistical variation from one another in terms of major mineral content (Table 3.8) and could be grouped as one for the use in ration formulations for the various geographical regions in SA. This allows us to create 3 fodder groups namely - kikuyu, lucerne and mixed grass to use in ration formulation for horses.

In terms of major mineral ratios, the NRC (2007) give an ideal ratio Ca:P ratio for horses of 1.2 - 2:1. Of the fodder types investigated in this study kikuyu, lucerne and veld do not fall within this range and contain 0.595, 4.8 and 3.69 Ca:P respectively. If fed alone these fodder types would require supplementary Ca or P to correct this imbalance.

Ca:Mg ratios of 1.5 - 2:1 are recommended for horses (Johnson *et al.*, 2004), eragrostis, teff and veld fall in this range with kikuyu being below 1.5 and lucerne, mixed hay and sweetveld contain more than twice the Ca to Mg content.

Trace mineral content varies between fodder groups and Pitzen (2006) reports average Fe content of 431 mg/kg in fodder with 10% containing over 1000 mg/kg in 3000 fodder samples analysed at Maddy Feed laboratories in a single year while Suttle (2010) gives Fe content between 70 to 3850 mg/kg in New Zealand and Australian pastures. Suttle (2010) reports Cu content of fodder at 4.5 to 21 mg/kg and pasture Zn levels between 25 – 50 mg/kg while Mn content varies widely with a mean of 86 mg/kg. Results from this study show similar trends in trace mineral content with high Fe (329 mg/kg), low Cu (5.65 mg/kg) and Zn (26 mg/kg) while Mn shows slightly higher average concentrations than those of Suttle (2010) at 102.6 mg/kg. As found in other countries the mean Fe content in SA fodder is far in excess of the 40 - 50 mg/kg recommended by the NRC (2007) and Cu and Zn are deficient when compared to horse requirements.

The ratio of Fe:Cu in the fodder analysed is far in excess of the recommended 10:1 ratio (Johnson *et al.*, 2004; Kellon, 2008a) which can cause imbalances in trace mineral uptake and status. Poor vaccine response is found in cattle with a Cu deficiency (Arthington *et al.*, 2002) and immune function impairment occurs in other species with poor Cu and Zn status (Larson, 2005). Larson (2005) reports marginal deficiencies of trace minerals are problematic as they are not manifested visually and producers are seldom aware of their existence. This situation could be problematic in SA where African Horse Sickness is endemic and poses a high risk of fatality for horses and could be exacerbated by trace mineral deficiencies. Improvement of trace mineral intake by supplementation of Cu and Zn to counteract high Fe intakes from fodder, may provide better immunity from vaccinations and improve the immune response in horses exposed to the virus. Where fodder and concentrate offered together, as they are in the horse, the problem of mineral intake, and mineral ratios, is exacerbated.

3.5 Conclusions

By generating nutrient norms for the different fodder types, feed formulations can be improved to prevent over and under provision of nutrients to horses. Mineral profiles of the fodder types can supply valuable information to nutritionists in determining the amounts of supplementary minerals needed in feeds, allowing them to provide rations with correctly balanced mineral content for horses.

Chapter 4 *In vitro* Feed Analysis

4.1 Introduction

Sales of horse feed from SA African Feed Manufacturers Association (AFMA) registered companies increased from 19643 t in 2006 to 38 999 t in 2015 (AFMA, 2015). This is a substantial increase, with sales more than doubling in the past nine years. Together with this there are several producers who are not registered with AFMA whose sales figures will not be reported and could raise these tonnages substantially. The horse feed market is highly competitive with various marketing strategies being used to win horse owners over to various brands with promises of increased performance, better health and improved behaviour resulting from correctly balanced feeds. The average horse owner understands little of the correct approach to scientific feeding principles and is lured by the promises offered in the various marketing strategies. If they perceive a shortfall in the nutrition of the horse they tend to increase the amount of feed a horse receives or find other supplements and feeds to add the diet without actually understanding what they are feeding or what the horse may be lacking.

Horses are generally fed a proportion of fodder and concentrate in their daily ration, depending on several factors predominantly influenced by work rate (Young, 2011). The proportion of nutrient dense, concentrated feed in a ration increases with the workload of the horse as the fodder fails to provide sufficient nutrition for higher work rates (Hussein and Vogedes, 2003). Fodder should make up the bulk of the ration with a minimum fodder intake of 1% of BW being recommended by Hintz (2000) while Coenen *et al.* (2011) have increased this to 2% BW in the latest German recommendations. Meyer (1999) stated "Our challenge is to develop species-specific, health-promoting nutrition to ensure a long, well-fed life." Pagan (2000) insists that supplementary grains, proteins and minerals given to horses must only supply essential nutrients not contained in the fodder. This shows that a supplementary feed should be formulated to provide the balance of nutrients not provided by the fodder component of the ration and should be fed at rates conducive to maintaining gut-health and wellbeing of the horse with sufficient fibre provision from fodder.

The NRC (2007) provides nutrient recommendations for life stage and work classes for horses. These recommendations are the bare minimum amount needed to prevent frank deficiency symptoms and make no provision for nutrient interactions or reduced absorption (Pagan, 1998). Mineral requirements should be inflated to at least 150% of the NRC values to ensure adequate nutrition for all classes of horse and to account for nutrient interactions within the ration (Kellon, 2008a; Pagan, 2009).

Horse feeds in SA are marketed predominantly on their CP content (Table 4.1) with energy and mineral content as secondary considerations.

Table 4. 1 Basic description of horse feeds available in the South African market showing the range of protein content of the different feeds (adapted from Young, 2011)

FEED	BASIC DESCRIPTION	PROTEIN %
STUD	Broodmare / growth	14, 16
RIDING -WORK	Grain	9, 10, 12, 13, 14
COOL	Grain free / cool	10, 11, 12, 14, 15
RACE	Thoroughbred racehorses in training	12, 14, 16
FIBRE	Conditioners/ fodder replacers	9, 10, 14, 15
VETERAN	Veteran / Golden	13, 14
SUPPLEMENTS	Balancer	20, 25

It is necessary to determine to what extent feed formulations are grouped in the horse concentrate feed categories and if the feeds are distinguishable from one another in terms of nutrient content. If feed groups vary in their nutrient content, it is possible to match feed and fodder profiles for optimal nutrient provision for the different classes of horse. As disparity exists between feed contents and feed bag labels (Hackland, 2007; Young, 2011) it is important to understand the extent of this difference in terms of macro-nutrient and mineral provision in order to match feed and fodder profiles to provide overall balanced rations.

The study evaluated 64 concentrate feed samples for nutrient content and used a statistical technique to determine horse feed classes. It was hypothesised that clear life stage feeding goals would inform clear feed categories per life stage and work type for horse concentrate feeds sold in the country.

4.2 Materials and Methods

4.2.1 Chemical analysis

Between April 2011 and May 2015, 64 feed samples of commonly available horse feeds were collected from around SA from horse owners and stable yards. Grab samples were taken from various bags of feed to ensure homogeneity of samples and over the course of the collection period certain feeds were sampled more than once from varying batches of the same feed.

Feed samples were submitted to the Cedara Feed Laboratory in KwaZulu-Natal, South Africa for proximate and chemical determination.

Samples were weighed and dried then milled through a 1 mm sieve in preparation for analysis. The chemical characteristics were determined as follows - DM % was obtained by drying of samples overnight in an oven at 100°C, Fat content was determined by the ether extraction method (AOAC 920.39), Ash content was determined (AOAC 942.05). ADF and NDF fractions were determined using the van Soest method on a digestion block (Van Soest *et al.*, 1991) and values were inclusive of residual ash. CP was calculated using the conversion factor of 6.25 (AOAC, 1990) after N was determined using the Dumas method (AOAC 698.06). Ca, P, Mg, Cu, Fe, Mn and Zn content was determined using the Hunter method (Hunter, 1984) and after digestion the minerals were read on an induction coupled plasma-atomic emission spectrometer (Varian ICP-OES Model MPX).

All figures were corrected to enable reporting on a 100% dry matter basis and results of the analyses were tabulated using Excel (MS Office 2013). Codes were assigned to each feed and for the factory producing them. DE was calculated by the following equation for concentrate feeds and protein supplements given by the NRC (2007).

$$DE\ (MJ/kg\ DM) = 4.184 \times (4.07 - (0.055 \times ADF\ \%))$$

NFE or nitrogen free extract which gives an indication of amount of soluble carbohydrates (starch and sugar) in the feed was calculated using the formula of Bredon *et al.* (1987) and Pagan (2000)

$$NFE\% = 100 - CP\ \% - Fat\ \% - NDF\ \% - Ash\ \%$$

NFE % calculations excluded ash as this was included in the Cedara NDF figures (Dugmore *pers. comm.*)

The matrix of nutrient analyses of the 64 horse feed samples was exported to Genstat v14 (2014) for further analysis.

4.2.2 Statistical analysis

In order to reduce multi-collinearity between classifying variables, a correlation matrix was produced. Classifying variables ADF, Ca, CP, DE, Fat, NDF and NFE were used for a PCA using a variance-covariance matrix to calculate PC scores to maximise nearest neighbour linkages. When high Principle Component (PC) scores were obtained indicating a high proportion of variation between samples, PCA Biplots were generated to elucidate the significance of the relevant classifying variables. Biplots were predictive with a Convex Hull to show distinct feed groups and the mean nutritional properties of the groups were then calculated to use as a basis for comparison.

The first PCA was used to discredit certain obvious outliers, before the balance of the more similar feed types were segregated on the basis of their chemical analysis. Excel (MSOffice 2013) tables were constructed for all feed groups. Treatment structures of feed category and factory were applied in an Unbalanced ANOVA to investigate differences in mineral content.

A simple correlation Excel (MSOffice 2013) between factory labels and the results of the *in silico* analysis above was used to ascertain compliance to predetermined feeding goals.

4.3 Results

Excel (MSOffice 2013) was used to calculate DE, NFE and Ca:P, Ca:Mg, Fe:Cu and Zn:Cu ratios and these figures were added to the spreadsheet of analysis figures. Feeds were allocated factory and feed codes and were classified into seven groups based on their marketed category :–

1. Balancer
2. Cool
3. Fibre
4. Leisure
5. Racing
6. Stud or breeding
7. Working

Classifying variables for use in PCA were identified and a correlation matrix was generated to reduce multi-collinearity between these classifying variables (Table 4.2). The high negative correlation between DE and ADF was expected as ADF is used in DE calculations. NFE shows large negative correlations to ADF and NDF indicating starch content drops as fibre content increase in feeds. DE shows a negative correlation to NDF and a positive correlation to NFE explaining the higher DE content found in rations with higher starch content and lower DE in high fibre feeds. The correlation between CP and Ash could result from the higher content of minerals occurring in stud and balancer feeds concomitant with higher protein content of these feeds.

Table 4. 2 Correlation matrix of 64 feed samples showing correlation coefficients between macro nutrients used as classifying variables in PCA

ADF	-					
Ash	-0.1807					
CP	-0.2896	0.7945				
DE MJ	-0.9295	0.1805	0.2755			
Fat	0.0062	-0.1548	-0.1091	-0.0154		
NDF	0.7933	-0.2757	-0.3657	-0.7240	-0.2343	
NFE	-0.7497	-0.0348	-0.0371	0.6806	0.1475	-0.9052
	ADF	**Ash**	**CP**	**DE MJ**	**Fat**	**NDF**

ADF %, CP %, NDF % and NFE % are effective in explaining 95.69% of the variation between feeds when used as classifying variables in a PCA. Eigen vector loadings (Table 4.3) making up PC score 1 (84.78%) show variation in feeds is primarily on the basis of NDF and NFE content while PC score 2 (10.91%) was differentiated on the basis of CP followed by NFE content with minor contributions from NDF and ADF. Feeds can therefore be differentiated from one another on the basis of their fibre and starch content while CP content accounts for a small proportion of variation in feeds as indicated by the disparity in PC score 1 and 2.

Table 4. 3 Eigen values making up latent vector loading scores indicating the contribution each of the classifying variables ADF %, CP %, NDF % and NFE % make to PC score 1 and 2 (95.69%)

	PC Score 1 (84.78%)	PC Score 2 (10.91%)
ADF	0.32556	0.15973
CP	-0.06854	-0.80372
NDF	0.70900	0.28505
NFE	-0.62179	0.49725

A PCA Biplot (Figure 4.1) was generated and showed two distinct groups of feeds apart from the rest namely - balancer and fibre feeds. Balancer feeds could be distinguished from the other feeds in terms of their high CP content which lies between 25 and 27% while the NFE, ADF and NDF content is low. Fibre feeds on the other hand are formulated on a fodder base with the addition of minerals and sometimes energy sources and are used as filler feeds to provide fibre in the diet. The fibre feeds had high ADF and NDF values and are differentiated into a distinct group by their fibre content. The protein content of the fibre feeds is similar to the other feeds as seen by the position of the group on the horizontal plane along with the other feeds. The NFE content is lower than in other feeds shifting the group’s position to the right of the main group.

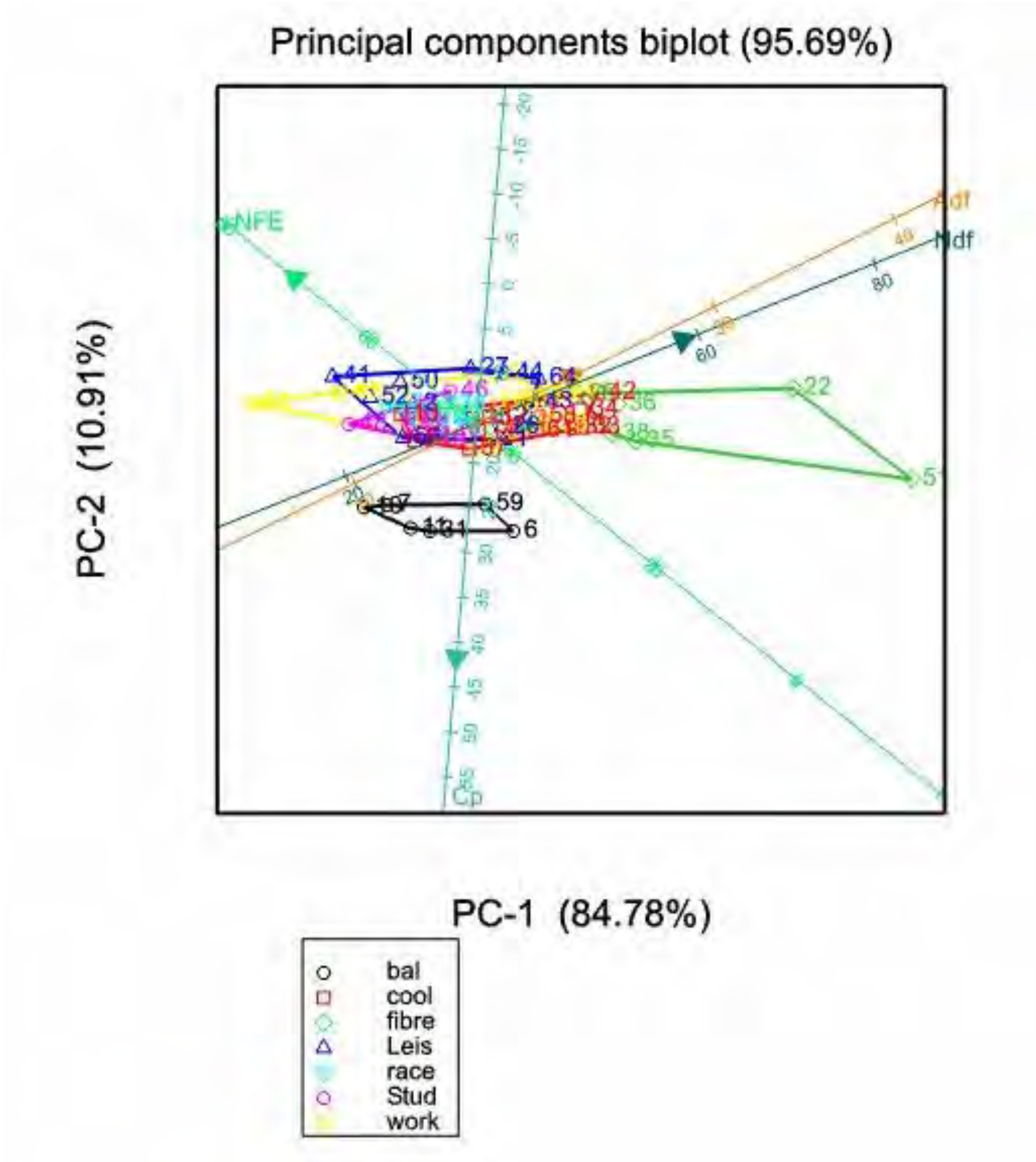


Figure 4. 1 Principal Component Analysis Biplot using ADF %, CP %, NFE % and NDF % as classifying variables conducted on 64 feed samples

The balance of the feeds occupy a space defined by the following ranges

ADF	15 - 28%
CP	10 - 17%
NDF	20 - 50%
NFE	40 - 50%

It was difficult to prise apart the 52 feeds that were not high CP balancer or fibre-based feeds. Using ADF, Ca, CP, Fat, NDF and NFE as classifying variables, upon which feeds should be discernible, two principle components were able to account for 91.61% of the variation in the sample set (Table 4.4).

Table 4. 4 Eigen values making up latent vector loading scores indicating the contribution each of the classifying variables ADF %, Ca %, CP %, Fat %, NDF % and NFE % make to PC score 1 and 2 (91.61%)

	PC Score 1 (83.32%)	PC Score 2 (8.286%)
ADF	-0.36183	0.80877
Ca	0.00991	0.01680
CP	0.06160	0.24270
Fat	0.01025	0.27598
NDF	-0.69262	-0.45430
NFE	0.62077	-0.06437

PC score 1 (83.32%) was determined by NDF followed by NFE then ADF indicating the variation between feeds is determined primarily by fibre and starch content (Table 4.4). PC Score 2 (8.28%) was strongly influenced by ADF content then NDF and CP and Fat contributed similar loadings to the score. Once again fibre is a primary variable giving variation in feeds while CP, on which feeds are formulated and sold, makes minor contributions to differentiation between feeds. That so much of the variation is captured by PC score 1 also indicates that little latitude is enjoyed in the nutrient contents of the feeds, given that the classifying variables provided the most significant over-arching principles by which the rations should be discerned. It is significant to note that the basis upon which commercial feeds are sold (CP %) figures as a small (0.24270) proportion of a very small loading score (8.286%).

The PCA Biplot (Figure 4.2) shows work and cool rations formed two very large groups with a wide spread indicating vast variations between the feeds making up these two groups. Cool feeds occupied a range to the left of the plot with a group of these having low NFE content compared to the bulk of the feeds - as cool feeds they are designed to contain less starch than standard working rations which can be seen in their position more to the left of the bulk of the working rations. The work group of feeds occupy a very large area indicative of their very diverse nutrient content. No standardised formulation goals for this group of feeds could be determined from this analysis.

Stud feeds form a smaller group to the right of the axes junction and extends slightly higher than some of the other feeds indicating their slightly higher CP, Ca and fat and lower NDF content compared to the bulk of the feeds. They have higher NFE content than many of the other feeds which shifts their position more to the right in the plot.

The racing rations form a tight group in the central lower right area of the plot. The small area occupied by the group indicates more uniformity in the nutrient content of these feeds.

Leisure feeds form a group on the lower edge of the cluster indicating they have lower levels of fat, CP, Ca and ADF but higher levels of NDF than many of the other feeds. The range of NFE is large and lies between 42 and 60%.

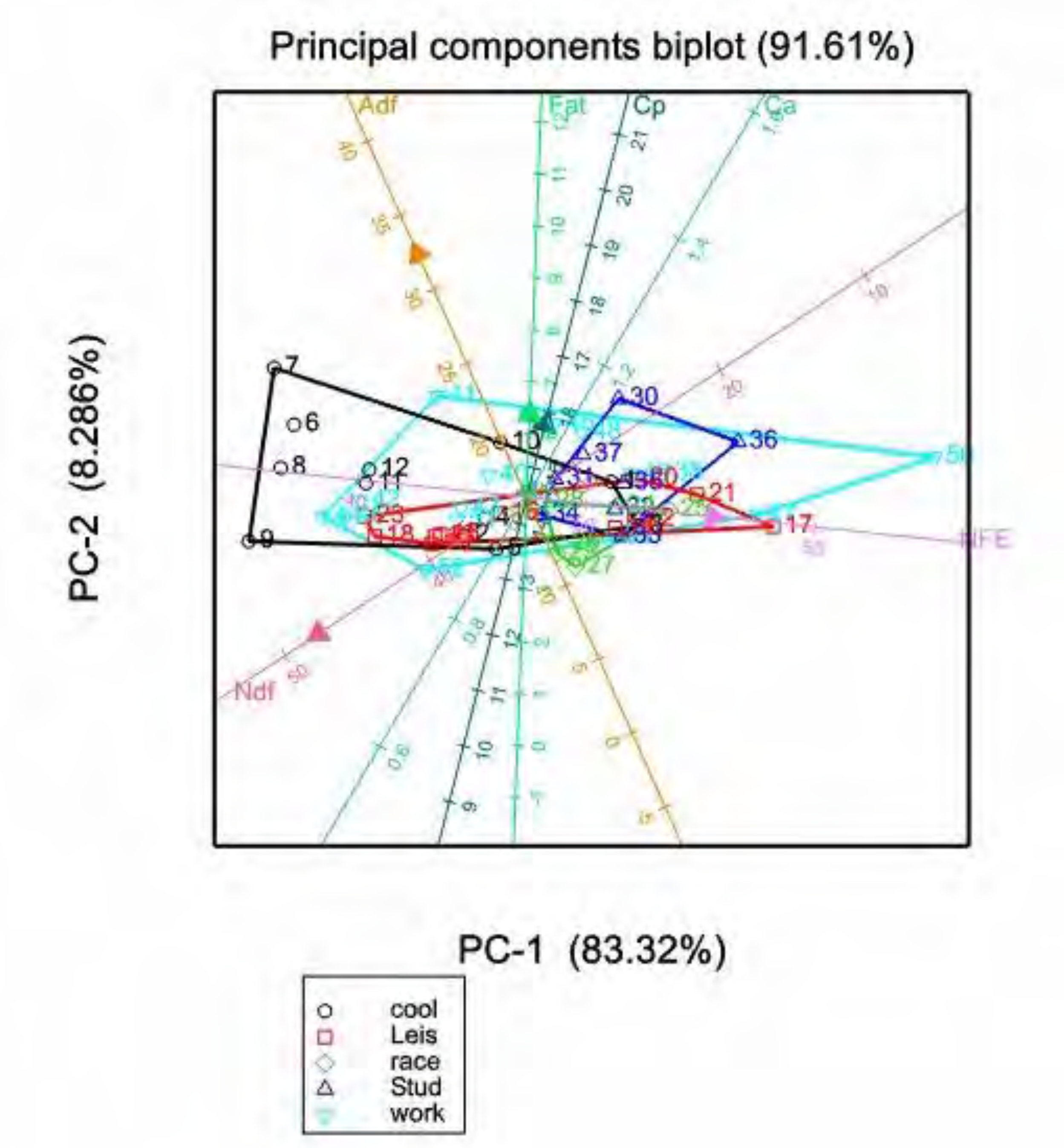


Figure 4. 2 Principal Component Analysis Biplot using ADF %, Ca g/kg, CP %, Fat %, NFE % and NDF % as classifying variables conducted on 52 feed samples excluding balancer and fibre feeds.

Using the 52 remaining samples, PCA's using Ca, Mg and P or Cu, Fe, Fe:Cu, Mn and Zn contents respectively were performed to analyse the effect of the mineral content, both major and trace, on the segregation of the horse feeds. Nominal categories as to how the feeds are sold were used merely as descriptors in the following two Biplots (Figure 4.3 & 4.4). The feeds for different purposes (work and leisure) and life stages (breeding and growth) should reflect the different purpose in the researched mineral levels appropriate to that purpose.

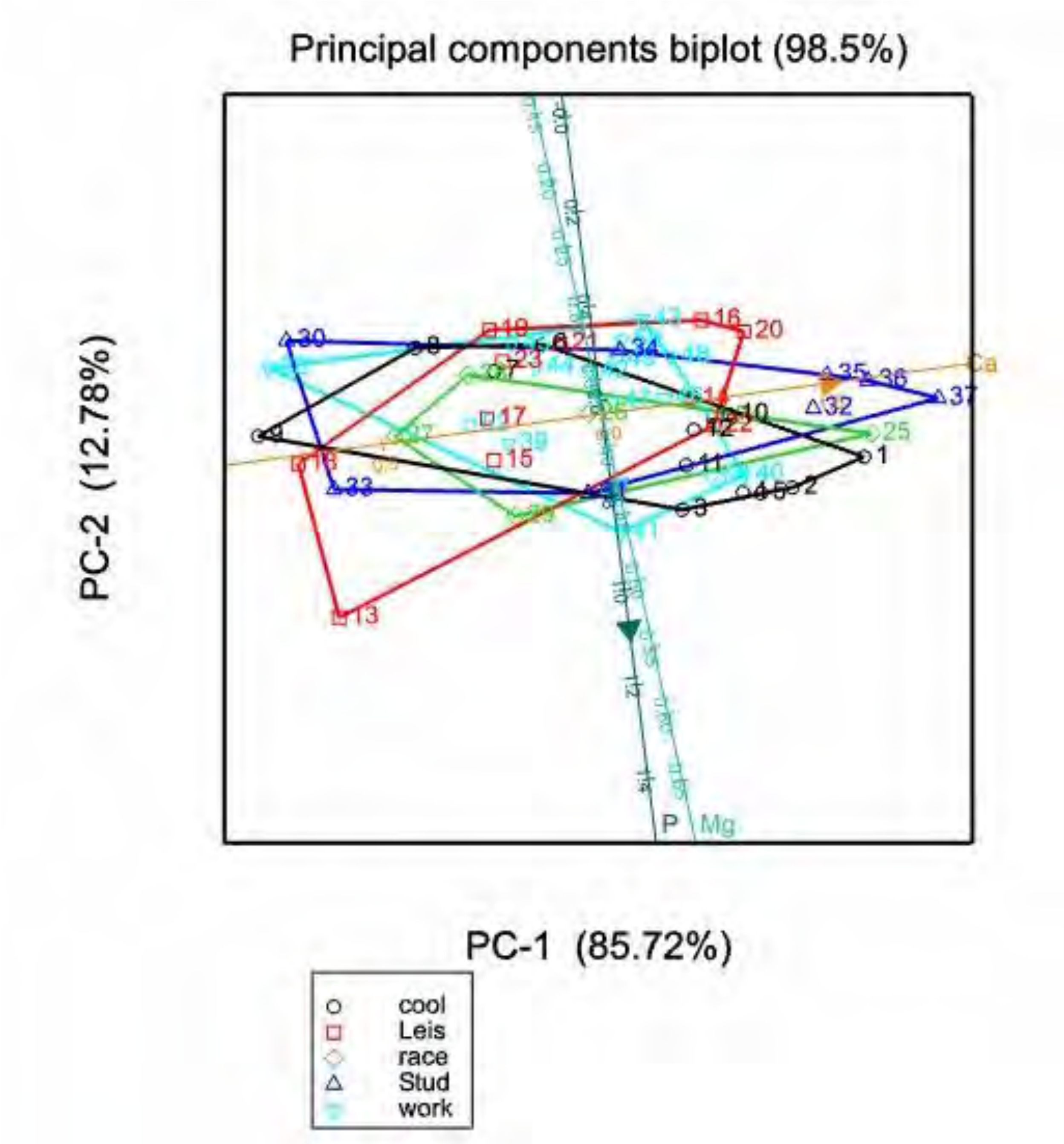


Figure 4. 3 Principal Component Analysis Biplot using major minerals Ca%, Mg % and P % as classifying variables on 52 feed samples excluding balancer and fibre feeds

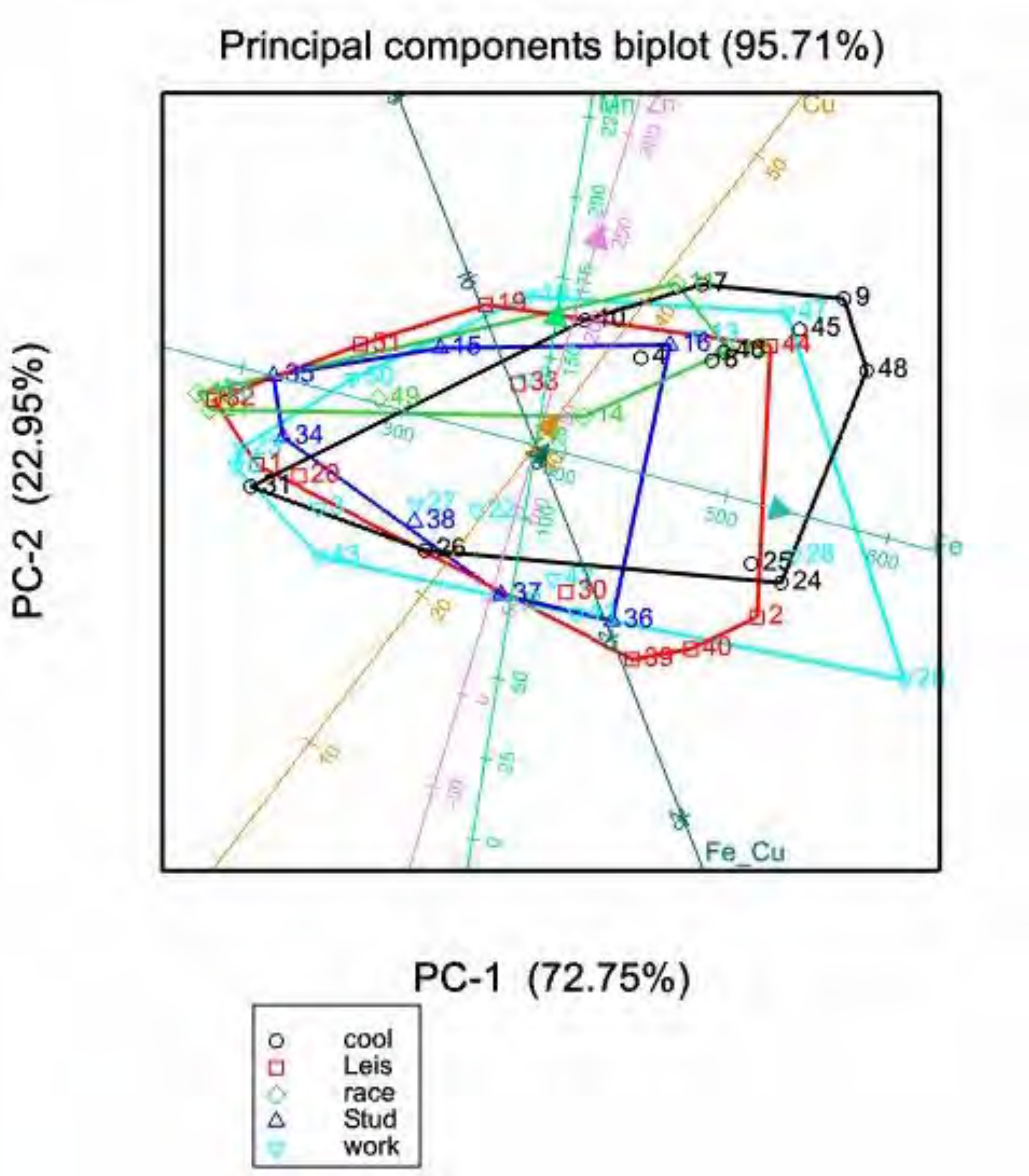


Figure 4. 4 Principle Component Analysis Biplot using Cu mg/kg, Fe mg/kg, Fe:Cu, Mn mg/kg and Zn mg/kg as classifying variables indicating large variation between 51 feed samples in respect of trace mineral content

It is evident from the figures that there is really no definite feeding goal or formulation goal in respect of major mineral content in the horse feeds as all the feeds occupy the same region of the plot. PCA's using Ca, Mg and P yielded a combined PC score of 98.5% made up of PC Score 1 (85.72%) with Eigen vector loading scores being heavily influenced by Ca content and PC Score 2 (12.78%) influenced primarily by P content then by Mg (Table 4.5). Again the large value attributed to PC Score 1 indicates Ca content of a feed is the most important factor accounting for the variation between feeds with little latitude offered by Mg and P in sample variation.

Table 4. 5 Eigen vector loading scores for PCA using Ca % , Mg % and P % as classifying variables and their contribution to PC Score 1 and PC Score 2 (98.5%) for 52 horse feed samples

	PC Score 1 (85.72%)	PC Score 2 (12.78%)
Ca	0.99045	0.13498
Mg	0.07054	-0.32217
P	0.11842	-0.93701

A PCA using Cu, Fe, Fe:Cu, Mn and Zn content as classifying variables produced a PC score of 95.81% indicating wide variation in trace mineral content in feeds. Table 4.6 shows PC Score 1 (73.29%) was heavily influenced by Fe content with Zn playing a secondary role while Cu, Fe:Cu, and Mn have a negligible effect on variation between samples. PC score 2 (22.52%) indicates Zn followed by Mn then Fe content of feeds account for some of the variation between samples. The PC Biplot (Figure 4.4) shows no separation of feed groups occurs on trace mineral content when feeds were categorised into their marketed categories.

Table 4. 6 Eigen vector loading scores for PCA using Cu mg/kg, Fe mg/kg, Fe:Cu, Mn mg/kg and Zn mg/kg content as classifying variables on 52 feed samples with two PC Scores (95.81%)

	PC Score 1 (73.29%)	PC Score 2 (22.55%)
Cu	0.05864	0.07303
Fe	0.95756	-0.27982
Fe : Cu	0.00965	-0.04654
Mn	0.09882	0.50887
Zn	0.26415	0.80948

It is evident however, upon analysis of the trace mineral values - that trace mineral levels are company dependant i.e. trace mineral content is determined by factory rather than formulation or feed goals (Figure 4.5). Higher Cu, Fe and Zn content in the ep and vit feeds move them to the upper right quadrant of the plot and the lower Fe content of afre group shifts their position to the upper left of the plot. Equu, alz, spur and gv occupy a lower position in the plot indicating they contain lower Cu, Mn and Zn content.

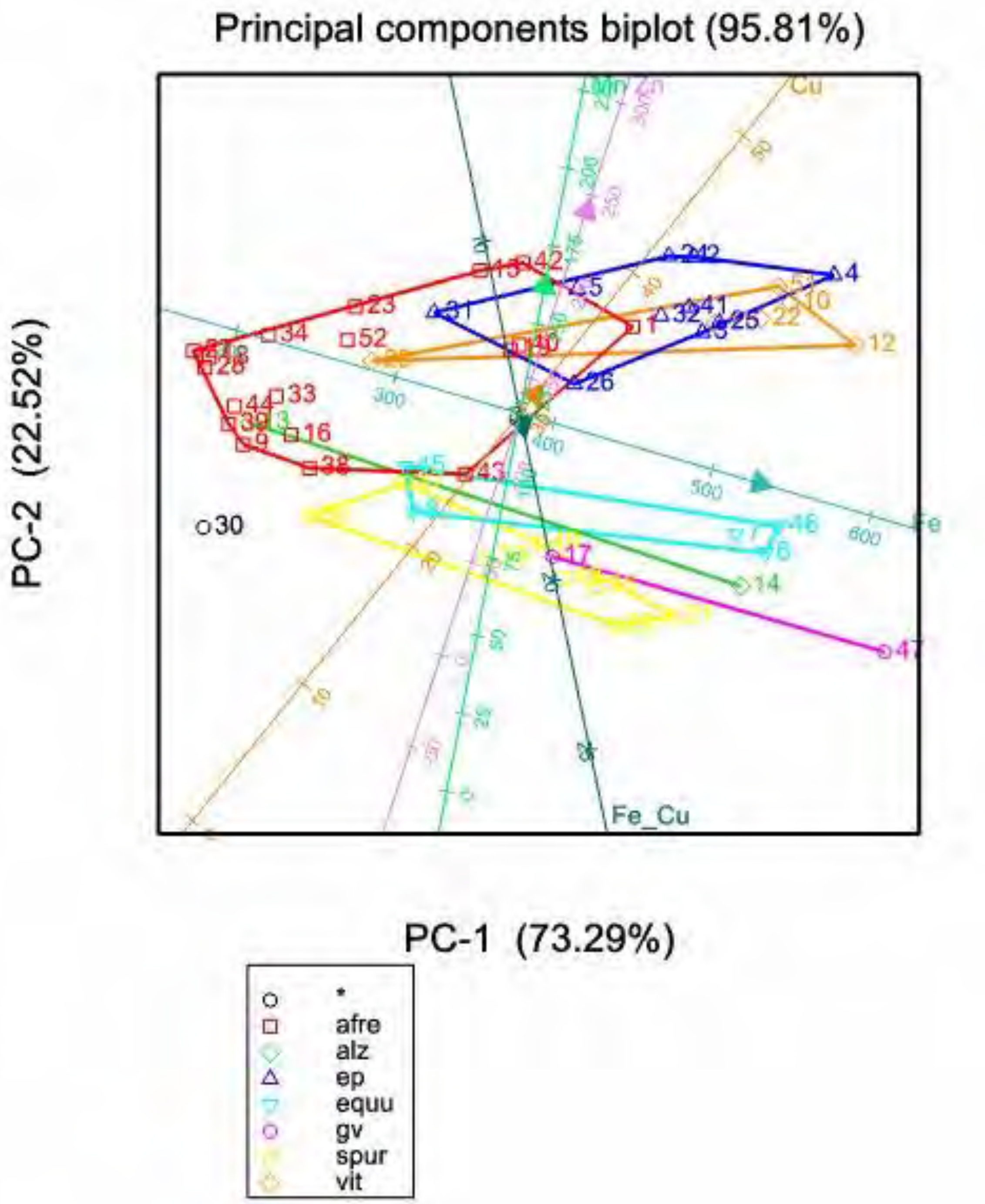


Figure 4. 5 Principal Component Analysis Biplot using Cu mg/kg, Fe mg/kg, Fe:Cu, Mn mg/kg and Zn mg/kg as classifying variables, where factories have been identified as sources of variation in feed samples

The Fe:Cu ratio has very little influence on the position of the feeds as indicated by the low Eigen vector loading score in Figure 4.5.The relatively large spread of the afre group can be partly accounted for by the large number of feeds analysed, representing 4 brands that are produced in the one factory. Alz and gv feeds show vast differences in the Fe content of the samples analysed as seen by the spread of the points in the plot.

The PCA could only segregate balancer and fibre feed groups from the 64 feed samples analysed. To obtain some generic contents for major nutrients, the most broad categories, not from the PCA, but from factories, were labelled as such in an Unbalanced ANOVA of the latter group of feeds in Genstat v14 (2014). One feed of unknown origin was excluded from the sample group. Significant differences ($P < 0.001$) occur in the mineral content of the feeds when categorised by factory with Vit having consistently higher mineral content than many of the other factories Table 4.7.

Table 4. 7 Mean nutrient content (100% DM) of 51 horse feeds excluding balancer and fibre groups when categorised by factory showing significant differences in nutrient content occurring as a function of factory

	n	ADF %	CP %	Fat %	NDF%	NFE %	Ca g/kg	P g/kg	Mg g/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg
Afr	18	16.37^{ab}	13.05	4.18^{ab}	36.74^{a}	46.03^{b}	0.794	0.587^{ab}	0.332^{bc}	27^{ab}	273^{b}	137^{ab}	117^{b}
Alz	2	13.25^{b}	15.26	4.2^{0ab}	34.12^{abc}	46.43^{b}	0.772	0.799^{a}	0.433^{ab}	17^{b}	381^{ab}	109^{bc}	73^{b}
Ep	10	14.43^{b}	15.35	4.22^{ab}	32.23^{abc}	48.20^{b}	1.232	0.774^{a}	0.424^{ab}	30^{ab}	438^{ab}	137^{ab}	223^{a}
Equu	5	23.27^{a}	14.55	6.47^{a}	39.89^{a}	39.09^{b}	0.839	0.489^{b}	0.349^{bc}	27^{ab}	455^{a}	93^{bc}	97^{b}
Gv	2	10.27^{b}	12.90	3.59^{b}	23.50^{bc}	60.00^{a}	0.8779	0.604^{ab}	0.368^{bc}	23^{b}	527^{a}	81^{bc}	66^{b}
Sp	8	14.04^{b}	15.56	5.46^{ab}	25.05^{bc}	53.93^{ab}	1.292	0.556^{b}	0.341^{bc}	28^{ab}	406^{ab}	67^{bc}	62^{b}
Vit	6	15.61^{ab}	15.62	6.01^{ab}	34.81^{ab}	43.55^{b}	1.139	0.704^{a}	0.494^{a}	52^{a}	475^{a}	175^{a}	197^{a}
Mean	51	15.85	14.42	4.81	33.48	47.29	1.01	0.63	0.38	30	382	123	133
SED		3.81	1.92	1.26	5.44	4.56	0.30	0.11	0.42	13	89	29	31
LSD (max)		7.67	3.88	2.54	10.97	9.19	0.61	0.22	0.08	26	179	58	63

$^{a\ b}$ Differing superscripts indicate significant differences between values in the same columns (P<0.001)

Consequently, the macro nutrient and mineral content for these same arbitrary life stage groupings were subjected to an Unbalanced ANOVA in Genstat v14 (2014) and the mean values are reported in Table 4.8 & 4.9

Table 4. 8 Mean nutrient content of 64 horse feeds (100% DM basis) used as classifying variables in PCA using marketed feed categories as determined by ANOVA using Genstat v14 (2014).

Feed Category	*n*	ADF %	Ash %	CP %	DE MJ/kg	Fat %	NFE %	NDF %
Balancer	6	13.06 ^{c}	19.08 ^{a}	26.91 ^{a}	14.02 ^{a}	3.26 ^{bc}	42.84 ^{b}	26.99 ^{c}
Fibre	6	27.56 ^{a}	9.92^{b}	13.91 ^{bc}	10.69 ^{b}	3.60 ^{bc}	26.95 ^{c}	55.54 ^{a}
Cool	12	18.73 ^{b}	9.42 ^{b}	14.92 ^{bc}	12.96 ^{a}	5.51 ^{a}	41.81 ^{b}	37.73 ^{b}
Leisure	11	15.02 ^{bc}	8.07 ^{b}	13.08 ^{c}	13.57 ^{a}	4.13 ^{abc}	48.83 ^{ab}	33.97 ^{bc}
Race	6	12.30 ^{c}	8.21 ^{b}	14.61^{bc}	14.20 ^{a}	4.24 ^{abc}	49.81 ^{ab}	31.33 ^{bc}
Stud	8	14.86 ^{bc}	8.94 ^{b}	16.24 ^{b}	13.61 ^{a}	4.36 ^{abc}	50.83 ^{a}	28.57 ^{c}
Work	15	16.43 ^{bc}	8.59 ^{b}	14.15 ^{bc}	13.25 ^{a}	5.12 ^{ab}	48.00 ^{ab}	32.72 ^{bc}
Mean	64	16.76	9.77	15.59	13.22	4.53	45.05	34.84
S.E.D.		2.32	1.35	1.21	0.63	0.80	3.88	4.30
LSD (max)		4.64	2.71	2.42	1.26	1.61	7.78	8.61

$^{a\,b}$ Differing superscripts indicate significant differences between values in the same columns (P<0.001)

Table 4. 9 Mean mineral content of 64 horse feeds (100% DM) grouped as per marketed feed categories

	n	Ca %	Mg %	P %	Cu mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg
Balancer	6	3.83 ^{a}	0.80 ^{a}	1.71 ^{a}	155.40 ^{a}	1190.00 ^{a}	399.30 ^{a}	525.30 ^{a}
Fibre	6	0.80 ^{b}	0.35 ^{b}	0.43 ^{c}	16.61 ^{b}	446.40 ^{b}	105.60 ^{b}	68.95 ^{c}
Cool	12	1.07 ^{b}	0.42 ^{b}	0.68 ^{b}	37.51 ^{b}	455.40 ^{b}	142.90 ^{b}	172.60 ^{b}
Leisure	11	0.87 ^{b}	0.36 ^{b}	0.60 $^{b c}$	24.44 ^{b}	369.10 ^{b}	120.20 ^{b}	108.90 $^{b c}$
Race	6	0.97 ^{b}	0.39 ^{b}	0.66 ^{b}	28.35 ^{b}	327.50 ^{b}	120.00 ^{b}	169.20 ^{b}
Stud	8	1.12 ^{b}	0.35 ^{b}	0.63 ^{b}	25.62 ^{b}	322.80 ^{b}	104.50 ^{b}	103.50 $^{b c}$
Work	15	0.96 ^{b}	0.35 ^{b}	0.59 $^{b c}$	30.13 ^{b}	374.50 ^{b}	114.70 ^{b}	114.10 $^{b c}$
Mean	64	1.24	0.41	0.71	40.30	461.10	146.00	162.40
SEM		0.34	0.06	0.10	11.61	135.90	29.23	41.74
LSD (max)		0.68	0.12	0.20	23.24	272.10	58.53	83.57

$^{a\ b}$ Differing superscripts indicate significant differences between values in the same columns (P<0.001)

It is expected that some random deviation from the mean of formulated, and therefore advertised, nutrient values should occur. A correlation matrix was set up in which advertised figures per brand were compared to the actual analysed contents of CP, Fat, Ca, P and Ca:P (min) of the feeds (Table 4.10).

Table 4. 10 Correlation coefficients obtained by comparing results of the feed analysis figures to figures advertised by horse feed factories

BRAND CODE	CP	FAT	CA (MIN)	P	CA:P (MIN)
CAP	0.954	0.949	0.773	0.983	0.799
EP	0.981	0.506	0.911	-0.916	
EQF	0.419	-0.058		0.155	
EQUU	0.959	0.788	0.970	0.989	-0.433
ROM	0.718			0.162	0.310
SP	0.906	0.725		0.792	
VIT	0.935	0.954	0.978	0.952	0.956
OVERALL	0.876	0.693	0.853	0.707	0.046

Correlations between advertised content and results from the feed analysis showed a correlation coefficient of 87% for CP, while fat showed a 69% correlation to label content. Minimum Ca values had an 85% correlation to advertised content while P showed a correlation of 71% to advertised content. When correlations were grouped by brand there were very large discrepancies between tested and advertised content of nutrients in certain brands while others showed consistently high correlations between tested and advertised content of nutrients as seen in (Table 4.10). Correlations were consistently poor for Eqf and Rom which are manufactured in the same factory. That Equu should have a very high correlation for Ca and P but not for the Ca:P ratio indicates an oversight in formulation strategy.

It is imperative that feeding goals and strategies be reflected in the feed in the bag. Of particular importance in this study was the way in which feed groups for life stage and purpose did not provide appropriate mineral contents, which includes balance between minerals known to interact with one another. A quick survey of the important mineral ratios (Table 4.11) shows imbalances occurring in feeds in respect of all the mineral ratios investigated.

Table 4. 11 Numbers of analysed horse feeds showing deviations from the ideal mineral ratios (n=64)

	CA : P	CA : MG	FE : CU	ZN : CU
TARGET RATIO	1.2 - 2 :1	1.5 - 2 :1	3 - 10 :1	3 - 4 : 1
LOW	15	7	1	17
IDEAL	30	7	20	13
HIGH	18	50	43	34
RANGE	0.43 - 4.43	0.70 - 7.44	4.00 - 47.43	1.30 - 14.84

4.4 Discussion

Feed formulation goals and strategies might be in place in the different feed companies, as seen by the advertised differences in the various feeds being produced. Analysis of the feeds shows very little evidence of how these strategies are implemented or what these strategies are. Besides balancer and fibre feeds, the majority of horse feeds do not differ from one another in terms of their chemical analysis and cannot be segregated into life stage or work groups, showing lack of formulation objectives. The same situation was found by Young (2011) where 11 classifying variables failed to allow the categorisation of SA horse feeds into their marketed category. In fact it was deduced that SA horse feeds are only differentiated on the basis of nutrients that are not formulated for. Hence feeds that are bought for differing life stage and purpose are different only inadvertently and not by any design or strategy of the factory or equine nutritionist (Young, 2011).

Balancer feeds provide high a content of protein and minerals with low starch inclusions and fulfil their role as nutrient dense feeds designed to complement high fodder intakes or raw materials when fed at low intakes (KER, 2015). Likewise, the fibre feeds which are fodder based feeds with limited raw ingredient additions, show high ADF and NDF content and low starch content. The formulation goals of both these feed groups appear to be met and the feeds are providing what they are designed to in terms of nutrient provision. This could simply be as a result of a limited raw material selection and very simple feed design brief.

The inability to segregate the balance of the feeds into life stage and work class groups raises questions about the formulation goals and strategies used in their production. It is obvious that the intention of feed manufacturers to provide feeds of varying nutrient content for the different life stage and purpose is not being carried through to the final product. Fradinho *et al.* (2006) is of the opinion that traditional horse diets often show mineral disparities, especially in terms of Ca, Mg and P provision. Owners are led to believe that manufacturers provide feeds offering optimal nutrition to horses while the opposite appears to occur and feeds appear to be formulated to the least cost objective as found by Young (2011). Horse riders also detect the inconsistency in the performance of horses as they manifest responses to inconsistent formulation goals. If feeds provided the nutrients that were missing from the fodder portion of the diet in a balanced manner, nutritionally, the horse then has the potential to meet the performance standards required of it.

The high NFE content in the majority of the analysed feeds provides empty calories in terms of nutrient provision and the horse will be oversupplied with energy while being deficient in fibre and minerals when fed the majority of the analysed feeds. Vervuert (2009) revised maximum starch intakes to <1.1 g/kg BW/meal to reduce insulin responses in horses. The high NFE content in the feeds will increase the risk of horses developing colic, laminitis, EMS and insulin resistance (Hoffman, 2009). This scenario of risk will be exacerbated in horses fed a large proportion of concentrate in their rations as they will experience the negative consequences of high starch and low fibre intakes on hindgut function with a concomitant acidifying effect which may increase Ca excretion in the urine and increase Ca usage to buffer high acidity levels created by the diet (Topliff, 2006).

Race feeds contain the lowest ADF (12.3%) and third lowest NDF (31.33%) content which are similar to levels found by Hackland (2007) where race rations contained 11.0 - 12.8% ADF and NDF of 27.5 - 28.5%. NDF fraction shows a slight increase in the current study. The 2.0 - 2.5 kg of hay intake per day shown by Hackland (2007) is far below the minimum requirement of 1.2% of BW given by Ellis and Saastamoinen (2008) for growing racehorses in training and the lack of fibre and excess starch in the ration will have negative consequences for hindgut function and can cause physical and behavioural issues in horses when their need for fibre is not met (Hoffman, 2009; Coenen *et al.*, 2011). The high protein content of the race feeds can lead to an oversupply of protein to racehorses at the current feeding high rates in SA (Hackland 2007). Ellis and Saastamoinen (2008) gave protein intake for a growing horse in heavy work as 1091 g per day which would amount to 11% CP in the total ration. Currently race feeds provide 14.6% protein which at a feed intake of 7 kg (Hackland, 2007) would provide 1022 g of CP and the 2.5 kg fodder portion of the diet will add a further 140 g for eragrostis or 462 g if lucerne is fed. The CP requirement of adult racehorses is 863 g per day (Ellis and Saastamoinen, 2008) which will result in far greater overprovision of CP in these horses. This overprovision of CP could result in unimproved race times, increases in heat stress, respiratory distress from high ammonia levels in the stables, increased water intake and therefore urination. No benefit of feeding excess protein levels to young racehorses has been shown (Kellon, 2008a; Frape, 2010; Young, 2011). The mineral content of this group of feeds is also high which may cause overprovision of certain minerals in the rations. When coupled with the high feed intakes, any mineral imbalance in the ration will be magnified in the horse.

Stud rations should contain higher levels of minerals than the work and leisure rations to supply the increased needs of late gestation, lactation and growth (Freeman, 1984; NRC, 2007). The results of the analysis show stud feeds contain the highest Ca content but low Cu, Mn and Zn contents while Fe contents are high. With the importance of Cu and Zn in immunity

(Larson, 2005) and joint integrity (Lawrence and Pagan, 2005) one would expect this class of feed to contain higher levels of these minerals than the working rations. They have the highest NFE content of all the feeds and this is problematic if these rations are fed to growing horses where high carbohydrate inclusions on the ration have been shown to increase joint problems (Becvarova and Buechner-Maxwell, 2012) in yearlings and in broodmares where EMS is a risk factor (Hoffman, 2009).

The ratios of minerals in the feeds were compared to the recommended ratios of between 1.2 - 2:1 for Ca:P (NRC 2007), Ca:Mg between 1.5 - 2:1 (Kellon, 2008a), Fe:Cu of 4 - 10:1 (Johnson *et al.*, 2004; Kellon, 2008a) and Zn:Cu of 3 - 4:1 (Johnson *et al.*, 2004; NRC, 2007). The number of feeds with poor Ca:P ratios is concerning as the imbalance between Ca and P in the diet has far reaching consequences for horse skeletal integrity and health. While certain brands of feeds regularly contain appropriate or slightly high Ca:P ratios, the occurrence of 15 out of the 64 feeds with inverse Ca:P ratios has the potential to cause lameness and fractures in horses due to Ca deficiency (Caple *et al.*, 1982; Stewart *et al.*, 2010). Hackland (2007) analysed racing rations of meal and cubes and found Ca:P ratios above 3:1 for the 3 feeds analysed. It can therefore be inferred that formulations have been incorrectly produced over an extended time period and the findings in this study are not unique in this regard.

Fifty of the 64 feeds contain Ca:Mg higher than 2:1 placing them above the higher recommended levels of Ca:Mg for horses and other species (Johnson *et al.*, 2004; Kellon, 2008a). This imbalance could result in deficiencies of Mg relative to Ca and P which may lead to over excitability and nervous behaviour in horses. The number of calming and cool foods on the SA sport horse market show there is a large need for behaviour modifications in the sport horse industry. As Mg is known as a calming mineral, higher inclusions to bring it into balance with Ca in feeds may be beneficial in many instances where calming feeds have been prescribed.

The Fe:Cu ratio of between 4:1 and 10:1 and a Zn:Cu of 1:3 is suggested as acceptable by Johnson *et al.* (2004) and *Kellon* (2008b). 43 of the 64 feeds analysed contain more than ten times more Fe than Cu and only 13 contain the correct amount of Zn in relation to Cu. This may exacerbate EMS and IR (Nielsen *et al.*, 2012) in horses and may lead to inflammatory conditions and poor immune response to vaccinations and general poor immune function (Arthington *et al.*, 2002; Larson, 2005). As with fodder the provision of Fe in feeds is far in excess of horse requirements while Cu and Zn content are low in comparison. If feeds were formulated with the fodder imbalance taken into account, feeds should contain minimal Fe content and higher levels of Cu and Zn to ensure overall ration intakes of the trace minerals are more balanced. Therefore the trace mineral balance in feeds is not suited to fodder provision and horses are at risk of trace mineral deficiencies and toxicities as found by Horne (2015) where 70% of examined liver samples showed excess Fe content while 68% were deficient in Zn.

4.5 Conclusions

In the livestock industry, measurable production parameters such as carcass weight, milk yield and feed conversion rates can be used to judge a feed's performance. This allows farmers and livestock managers to choose feeds that maximise livestock production thus ensuring companies uphold strict standards of formulation to maintain their market share. In the horse industry, performance criteria are largely subjective and based on owner opinion with no measurable parameters being available on which to judge a feed's performance. This has allowed the horse feed companies to produce sub-standard feeds, which are often vastly oversupplied to horses, in the vain hope of improving their performance. The establishment of standardised testing and performance parameters and feed intake norms would greatly enhance the uniformity and quality of horse feeds, allowing owners to make informed choices as what feeds they use.

In addition, cognisance of these mineral imbalances will at least encourage nutritionists to put these parameters in their fed formulations. Near Infra-Red Spectrophotometry and advances in raw material testing must pass through to the horse industry. Horse nutritionists need to be aware of the dynamic nature of raw material specification fluctuations in their formulations. They need to appreciate the effect these have on the quality of the feed. Horse feed factories need to borrow some accountability and responsibility from the broiler and dairy industries.

Chapter 5 *In Silico* fodder and feed evaluation

5.1 Introduction

The use of metabolic modelling in horse nutrition would go a long way to improving the presentation of horse feeds and horse nutrition in SA (Young, 2011). From the results of Chapter 4 it can be seen that little evidence exists that feed formulation strategies are in place for the horse feeds currently produced in the country. Typically, horses are fed a set amount of concentrate feed and topped up with fodder, usually in the form of hay, with little attention given to the nutrients provided by the fodder. The amount of feed provided is generally work rate dependant and fodder type is primarily a function of geographical location and availability. Young (2011) gives typical concentrate: fodder ratios of SA rations as follows: idle - 20:80, sport horses - 50:50 and Thoroughbred racehorses in training 80:20. The NRC (2007) give DMI of horses as 2 - 2.5% BW which can be used to calculate the weight of fodder and feed that constitute the daily ration.

Feed formulation goals and strategies of the feed companies cannot be deduced from proximate and chemical characteristics of the feed as found in the previous Chapter and supported by Young (2011). At present, it appears that a least cost objective is used in formulation (Young, 2011) with high inclusions of carbohydrates. Coupled to this, the differing Vimi packs are obfuscated in the permutations of raw ingredients used in production of the feeds that have also not been minerally cognisant. Typically when unsatisfactory performance is perceived to occur, the concentrate portion of the diet is increased with little cognisance of the nutritional needs of the horse leading to an increase in feed sales rewarding the companies for poor formulations instead of holding them accountable for poor feed production.

In order to produce an effective feed formulation strategy, a metabolic model of the horse is needed to allow accurate requirements for breed, work, environment and life stage to be determined. Emmans and Kyriazakis (2001) (Figure 5.1) show actual feed intake is a function of many factors such as genetic potential and current state of the horse, taking into account the metabolic rates, environment and physical constraints such as gut capacity along with feed resources available in the form of raw materials. The principle of the first limiting nutrient will also come into play and has the potential to increase feed intake until the requirement for the nutrient is met or gut capacity is met (Young, 2011). At present feed intake in horses is currently used as an input in ration formulation instead of a performance driven output (Young, 2011). In order to effectively feed horses a deeper understanding of the factors affecting intake and requirements and their effect on the horse is necessary. This will allow horse nutrition to shift from a companion-animal based practice to a performance based one.

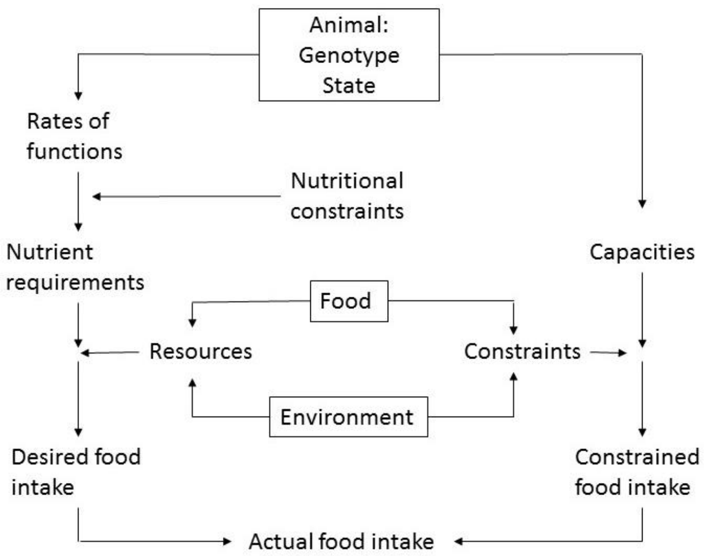


Figure 5. 1 A schematic representation of the theory that 'animals eat to meet their requirements subject to constraints' (Emmans and Kyriazakis, 2001)

Coenen *et al.* (2011) describe updated models of metabolisable energy and digestible protein which are being developed to define more accurately the energy and protein requirements for different breeds of horses, at different life stages and workloads. As more information becomes available it will be possible to formulate horse rations using these metabolic models taking into account all the factors shown by Emmans and Kyriazakis (2001). Presently metabolisable energy requirements given for certain horse breeds in moderate work and housed in box stalls as described in Figure 5.1. Factors such as training stage, body condition, group vs solitary housing, pasture access and environmental temperatures can change these requirements by up to 50%. As yet little information exists on pre-caecal protein digestion and mineral absorption to allow modelling of these nutrients in horse rations. Very little choice of breed specific feed is available on the SA market.

Updated requirements for maximum starch provision of 1 g per kg BW per meal and minimum roughage provision of 20 g per kg BW of fodder with 84 - 90% DM are given by Coenen *et al.* (2011) and will form part of the latest German requirements for horses currently being formulated.

A large paradigm shift is going to be necessary from both the feed companies and horse owners before the implementation of the new feeding strategies will be adopted. The mentality that horses cannot survive without large amounts of feed will have to be overcome and the shift from carbohydrate rich, least cost formulations to higher quality, lower starch, and fibre-rich rations will gain momentum when owners see the benefits in the horse. The production of balancer feeds has started the shift and their popularity shows the horses are responding to the higher nutrient levels positively. A benefit of these types of feeds is the ability to choose feeding levels of protein and minerals independent of energy provision making the feed more flexible for a wider range of fodder and horse types.

Hence, several scenarios have been combined in an *in silico* evaluation of ration composition. Feed (Chapter 4) and fodder types (Chapter 3) have been used to evaluate nutrient and, in particular, mineral provision to various classes of horses. The hypothesis is that current concentrate feeding regimes don’t complement the fodder nutrient provision in the meeting of daily nutrient requirements. Consequently, it is necessary to revise ration formulation goals given new evidence on starch and fodder inclusion rates (Coenen *et al.*, 2011).

5.2 Materials and Methods

Results from Chapter 3 (fodder) and Chapter 4 (feed) were used in an *in silico* scenario analysis of nutrient provision. Fodder types were assesses for their ability to provide minerals and nutrients, and then hypothetical horses were created to assess five feed and fodder combinations for nutritional adequacy. Sections 5.2.1 and 5.2.2 further elaborate the method for this analysis.

5.2.1 Fodder evaluation.

Mean nutrient content of the three fodder groups determined in Chapter3 (kikuyu, lucerne and grasses) (Table 5.3) were used in scenarios where daily rations were reconstituted for a 500 kg horse eating 2% of BW in fodder. NRC (2007) stipulations for nutrient intake were used as reference values in the generation of radar charts (Excel, MSOffice 2013). The total nutrient provision for each fodder evaluated dietary adequacy.

Assumptions were that a horse with a DMI of 2% was provided with 100% of DMI in fodder.

Nutritional adequacy was reflected by the radar charts, which should the percentage of requirement (NRC 2007) was calculated.

5.2.2 Feed evaluation

Although statistically, feeds could only be segregated in three disparate feed groups, Table 4.8Table 4. 8 gives mean nutrient and mineral content of the seven marketed feed categories used in this retrospective evaluation. Two feeds were used in the categories pertaining to the horses below (Table 5.1) and the mean nutrient content of that group of feeds was used as a group mean value in calculations for the horses.

Hypothetical horses were created for this exercise to encompass a range of life-stages and work types. The model horses are as follows:

1. 2 year old Thoroughbred racehorse in heavy training weighing 430 kg eating 2.1% of BW
2. An adult Warmblood in the first month of lactation weighing 600 kg eating 2.5% of BW
3. A 10 year old Sport horse showjumper in moderate work weighing 550 kg eating 2% of BW
4. Arab gelding used for light hacking weighing 400 kg eating 2% of BW
5. An overweight, aged pony with EMS weighing 200 kg eating 2% of BW

Table 5.1 outlines fodder and feed combinations for these five horses.

Table 5. 1 Feed and fodder make up of typical South African Horse rations showing dry matter intakes (DMI) as a percentage of BW (kg) given by NRC (2007)

HORSE	BW KG	DMI % BW	DMI KG	FEED KG / DAY	FODDER KG/DAY
2 YEAR OLD RACING THOROUGHBRED	430	2.1*	9	7 kg ERPM or EQFRP14	2 kg eragrostis
ADULT WARMBLOOD BROODMARE LACT 1 MONTH	600	2.5	15	7.5 kg ROMSF or SPBM	7.5 kg kikuyu
10 YEAR OLD SHOW JUMPER MOD WORK	500	2	10	5 kg EQFSF14, CAPPT, EQUCNP15 or VITC	2.5 kg lucerne + 2.5 kg teff
ADULT ARAB HACK	400	2	8	2 kg ALGV10 or VUMV10	6 kg of sweetveld
AGED PONY WITH EMS	200	1.75	3.5	0.5 kg EQUSNL or VITVET	3.0 kg sweetveld

* Figures taken from Hackland (2007)

5.3 Results

5.3.1 Fodder evaluation

To investigate the ability of fodder to support horses from differing work and life stage categories the NRC (2007) requirements for 500 kg horses (Table 5.2Table 5. 2) were compared to nutrient provision from the three fodder types assuming 100% fodder intake.

Table 5. 2 Minimum nutrient requirements of various classes of horses (mature body weight 500 kg) given by the NRC (2007)

HORSE CLASS	DE MJ	CP G	CA G	MG G	P G	CU MG	FE MG	MN MG	ZN MG
ADULT – AVERAGE MAINTENANCE	70	630	20	7.5	14	100	400	400	400
LIGHT WORK	84	699	30	9.5	18	100	400	400	400
MODERATE WORK	97	768	35	11.5	21	113	450	450	450
HEAVY WORK	111	862	40	15	29	125	500	500	500
PREGNANT MARE - 11 MONTHS	90	893	36	7.7	26.3	125	500	400	400
LACTATION - 1 MONTH	133	1535	59.1	11.2	38.3	125	625	500	500
GROWTH - 12 MONTHS	79	846	37.7	5.4	20.9	81	402	321	321
24 MONTHS VERY HEAVY WORK	136	1091	36.7	12.9	20.4	107	537	429	429

**No extra minerals provisions are made for growing horses in work by the NRC (2007)

Mean nutrient content of the three fodder groups determined to be statistically different from one another in Chapter 3 are given in Table 5.3.

Table 5. 3 Mean nutritional content (100% DM) of three South African fodder types which were determined to be statistically different from one another

FODDER	DE MJ	CP G	CA G	MG G	P G	CU MG	FE MG	MN MG	ZN MG
KIKUYU	10,4	203	1.6	1.2	3.2	9	322	144	32
LUCERNE	10,0	185	10.9	2.9	2.5	9	394	45	25
GRASSES	7.6	73	2.8	1.6	1.7	5	323	100	25

Radar charts were formed to show the differences in nutrient provision of 10 kg of fodder for the three fodder types. Figure 5.2 is used to show the ideal situation where nutrient provision of the ration meets 100% of horse requirements.

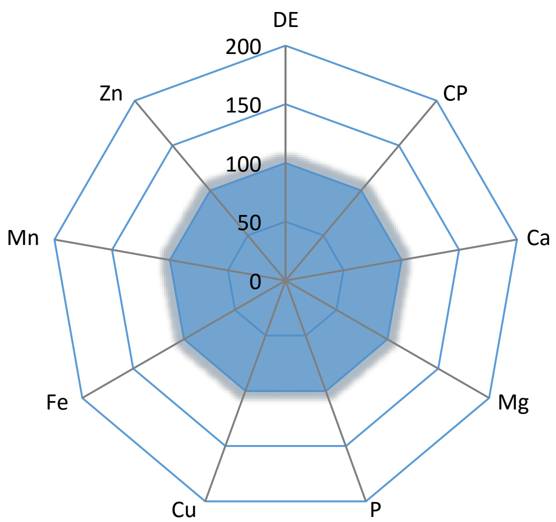


Figure 5. 2 Radar Chart showing the ideal situation where nutrient provision of a ration meets 100 % of horse requirements for all nutrients.

Idle adult horses have the lowest requirements of all the horse classes. Figure 5.3 shows the ability of each of the three fodder types to supply an idle 500 kg horse eating 2% of BW with its minimum nutrient requirements. Kikuyu and lucerne provide DE and CP in excess of horse requirements while grasses are close to 100% of requirements for an idle horse. Mineral provision of the three fodder types varies widely and mineral supplementation will be necessary in all three fodder types for all horse classes to ensure absolute mineral requirement is met and to balance mineral ratios. Cu and Zn fail to meet horse requirements in all the fodder groups and kikuyu is deficient in Ca and Mg but supplies excess P, Fe and Mn. Lucerne supplies excess Ca, Fe, Mg and P and is deficient in Mn. Grasses meet requirements for all but the Cu and Zn.

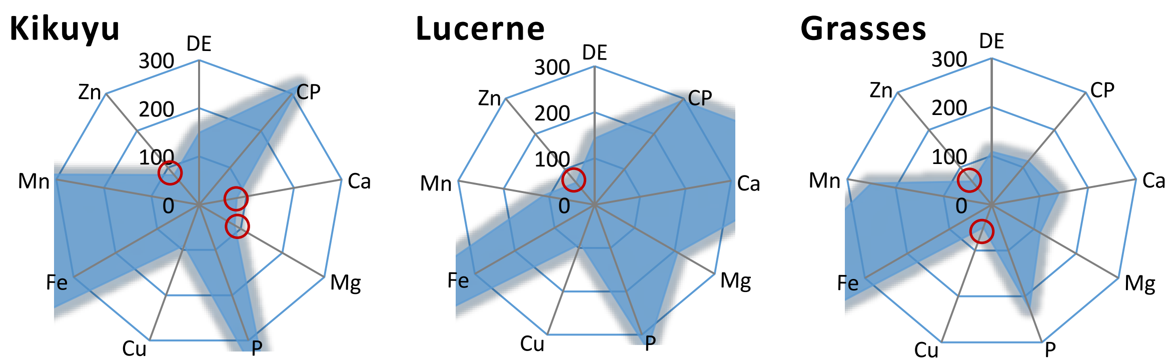


Figure 5. 3 Radar charts illustrating the nutrient provision of 10 kg of three fodder types expressed as a percentage of horse requirements for a 500 kg idle horse.

When the fodder nutrient provision is compared to requirements for a 500 kg horse in light work (Figure 5.4) lucerne and kikuyu provide sufficient DE and CP and show the same mineral deficiencies and excess as for idle horses. Grasses fall short of providing adequate DE, Ca and Mg for horse requirements and provide CP at 100% of requirement. As horse requirements rise above those of light work, the grasses group fails to meet requirements for both DE and CP necessitating the addition of an alternative feed source for all moderate work and breeding or growing horses eating grasses.

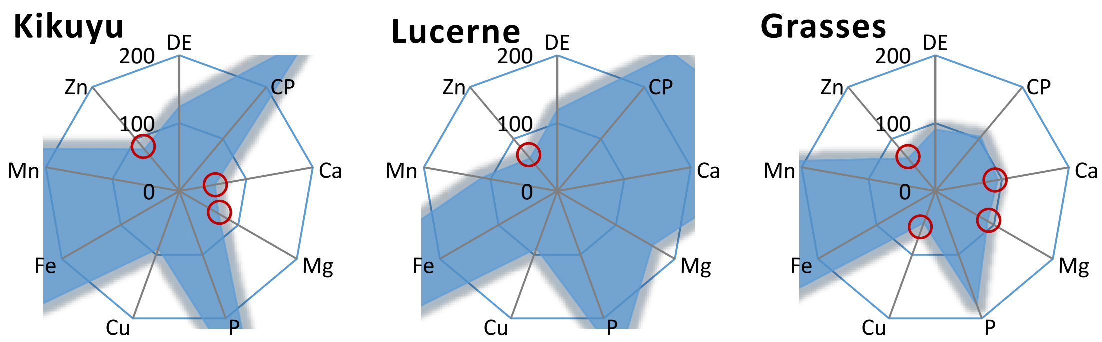


Figure 5. 4 Radar charts illustrating the nutrient provision of 10 kg of three fodder types expressed as a percentage of horse requirements for a 500 kg horse in light work.

When compared to requirements for moderate work (Figure 5.5), kikuyu and lucerne provide DE at close to 100% of requirements and CP provision remains above requirements. These two fodder types will therefore not meet horse requirements at levels above those of moderate work, i.e. those in hard work, growth or lactating brood mares and these classes of horse will need supplementary feed.

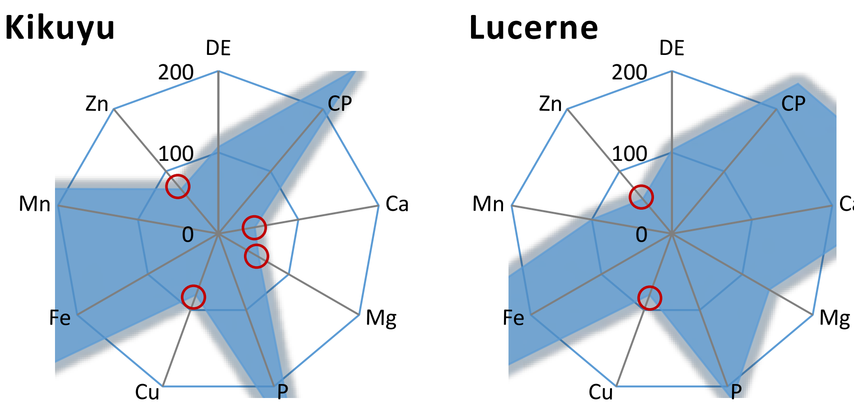


Figure 5. 5 Radar Charts showing the nutrient provision from 10 kg of kikuyu and lucerne expressed as a percentage of horse requirements for a 500 kg horse in moderate work.

Most significantly, this means that any horse doing and form of work needs supplementation, but more specifically Cu, Mg and Zn supplementation.

5.3.2 Feed evaluation

Energy, protein and mineral requirements for five horse scenarios are tabulated (Table 5.4)

Table 5. 4 Horse requirements (NRC, 2007) for DE and CP with mineral levels set to 150% of NRC recommendations except for Fe which remains at NRC (2007) levels

HORSE	DE MJ	CP G	CA G	MG G	P G	CU MG	FE MG	MN MG	ZN MG
2 YEAR OLD RACEHORSE	117	969	55	19	31	161	537	644	644
BROODMARE	160	1842	106	20	69	225	750	900	900
SPORT HORSE	107	768	58	19	35	186	495	743	743
HACK	67	559	36	11	22	120	320	480	480
GERIATRIC	28[a]	216	12	5	8	60	160	240	240

[a] NRC requirement. Actual requirement needs to be lower than this to allow weight loss in this profile of horse

a) Two year old racehorse in heavy work, weighing 430 kg eating 2.1% of BW

Nutrient content of a racing ration consisting of 7 kg of concentrate feed and 2 kg of eragrostis hay was calculated using two race feeds and the race group mean (Table 5.5). The three rations provide close to 100% of requirements for DE and slightly higher CP than required. Radar charts (Figure 5.6) show that the EPRM ration provides nearly 200% Ca with Mg, P, Cu and Mn supply being > 150% of requirements and Zn over 200%, Fe is oversupplied at 708% of requirements. In contrast, the EQFRP14 is deficient in Ca while Mg and P are slightly oversupplied between 100 and 150%. Cu provision is slightly lower than requirements and Mn and Zn provision is slightly higher than requirements and Fe is once again vastly oversupplied at more than three times the required daily intake. As this horse is still growing and needs special attention paid to Ca, Mg and P provision, the extra Ca, Mg and P provided by the EPRM will be beneficial in terms of mineral absorption and bone density. The EQFRP14 with the inverse Ca:P ratio will have devastating consequences in the macro-mineral provision in this horse.

Table 5. 5 Horse requirements and ration nutrient provision for a two year old racehorse, in heavy work, fed 2 kg of eragrostis hay and 7 kg of two selected race feeds and the mean race feed group (100% DM)

	DE MJ	CP G	CA G	MG G	P G	CU MG	FE MG	MN MG	ZN MG
REQUIREMENTS	117	969	55	19	31	161	537	644	644
EPRM	116	1143	115	33	55	282	3805	1162	1609
EQFRP14	117	1110	42	26	47	163	1698	882	916
MEAN OF RACE GROUP	115	1176	75	31	52	207	2744	882	1330

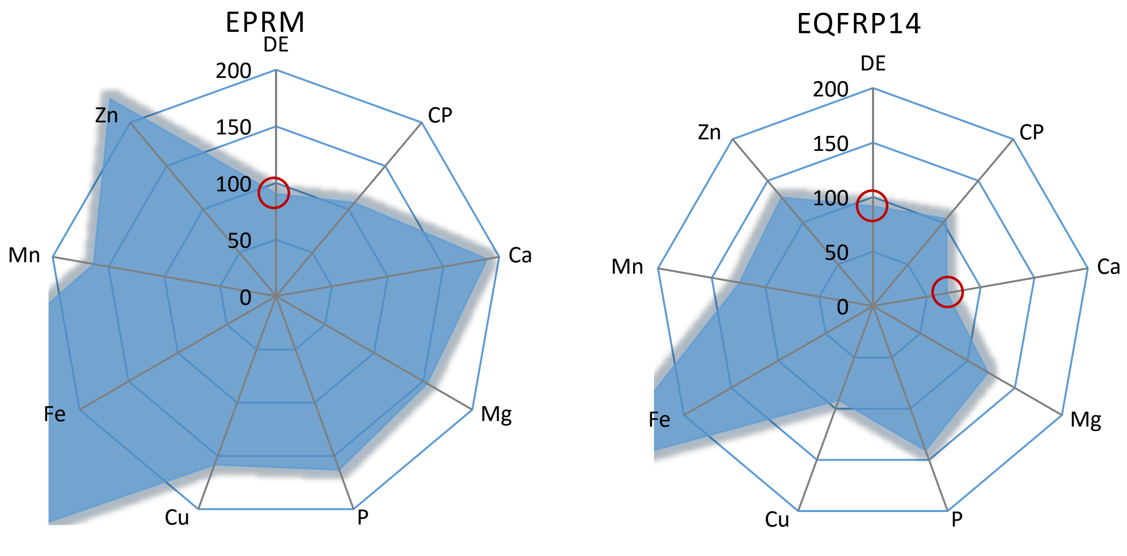


Figure 5. 6 Radar charts showing the percentage of nutrient requirements provided in the ration by two racehorse feeds fed at 7 kg per day with 2 kg of eragrostis hay

b) Warmblood broodmare in the first month of lactation, weighing 600 kg, eating 2.5% of BW

Fed a 50:50 ratio of fodder to concentrate this mare would receive 7.5 kg kikuyu (in most established breeding areas of the country) with 7.5 kg of concentrate – this would be a stud feed. Two stud feeds and stud group mean are shown relative to requirements in (Table 5.6).

Table 5. 6 Nutrient requirements and ration nutrient provision for a 600 kg broodmare in the first month of lactation fed 7.5 kg of feed and 7.5 kg kikuyu (DM) using two selected stud feeds and the mean of the stud group of feeds (100% DM)

	DE MJ	CP G	CA G	MG G	P G	CU MG	FE MG	MN MG	ZN MG
REQUIREMENTS	**159**	**1842**	**106**	**20**	**69**	**225**	**750**	**900**	**900**
ROMSF	184	2574	66	36	70	249	4129	2085	1043
SPBM	182	2852	137	34	75	283	5078	1725	724
STUD GROUP MEAN	182	2738	96	35	71	257	4844	1864	1016

Mineral provision of the two rations varies widely and the brand of feed makes a large difference in mineral provision to broodmares. While DE and CP are more than requirements - cutting the feed quantity would mean exacerbating the discrepancies in mineral provision (Figure 5.7). ROMSF does not meet Ca requirements but does meet requirements for P while CP, Mg, Fe and Mn are oversupplied. Zn and DE provision is slightly higher than requirements. SPBM shows a much more uniform supply of nutrients. DE provision is similar to ROMSF at over 100% while CP, Ca, Mg, Cu, Fe and Mn are oversupplied and Zn is slightly below requirements.

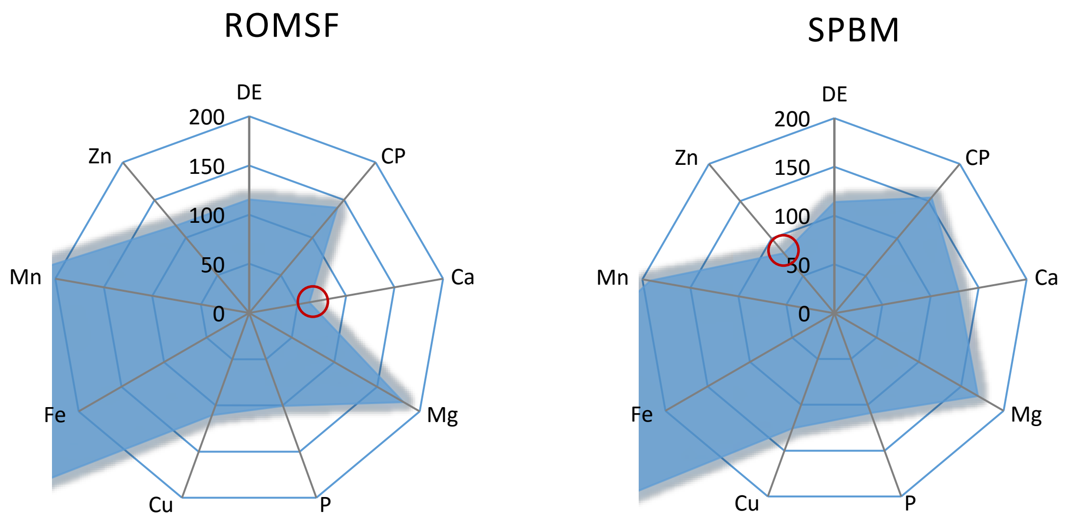


Figure 5. 7 Radar charts showing the percentage of nutrient requirements provided by two stud feeds fed at 7.5 kg with 7.5 kg of kikuyu fodder

c) Showjumper in moderate work, weighing 500 kg and eating 2% of BW

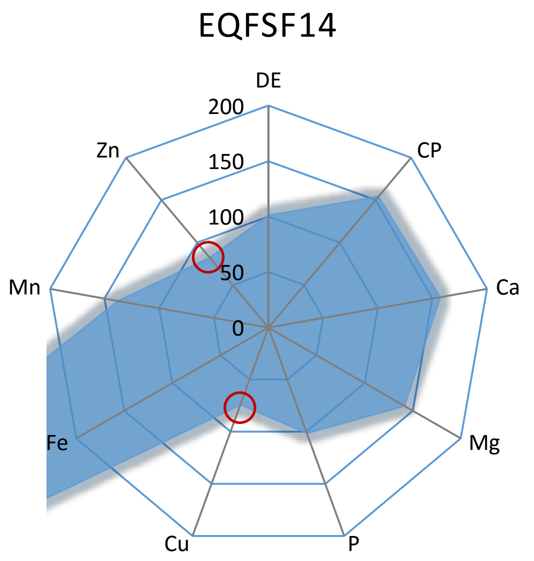
Feeds for working horses are provided in two forms depending on the type and form energy in the ration. Cool feeds reduce "hot" behaviour in horses as they contain low grain and higher fat inclusions. Starch is modified to allow greater SI digestion and less overflow into the hindgut.

Rations for the sport horse in moderate work were chosen from both cool and work groups of feed and rations consisted of 50:50 fodder to concentrate with 2.5 kg of teff and 2.5 kg of lucerne making up the fodder portion of the ration (Table 5.7). Energy provision in three of the four rations closely matches requirements.

Table 5. 7 Nutrient requirement and ration nutrient provision for a 500 kg sport horse in moderate work eating 5 kg of feed with 2.5 kg of teff and 2.5 kg of lucerne (100% DM)

	DE MJ	CP g	Ca g	Mg g	P g	Cu mg	Fe mg	Mn mg	Zn mg
Requirements	107	768	58	19	35	186	495	743	743
Work Group Mean	111	1390	84	30	43	193	3520	1059	743
EQFSF14	108	1186	91	27	36	138	3438	1025	613
CAPPT	118	1442	75	28	48	148	2703	970	578
Cool Group Mean	110	1426	89	34	47	233	3923	1200	1172
VITC	108	1450	96	37	47	346	4247	1444	716
EQUCNP15	96	1464	74	31	38	196	4270	955	1038

Figure 5.8 shows the overprovision of CP, Ca and Mg, in the rations using EQFSF14, with 150% of requirement being provided while P meets horse requirements. Cu and Zn are under provision with Mn supplied at 150% of requirements and Fe is oversupplied. CAPPT shows CP at 200% of requirements with Ca, Mg, Mn and P lying between 130 – 145% of requirements. Once again Cu and Zn are below requirements and a large oversupply of Fe occurs.



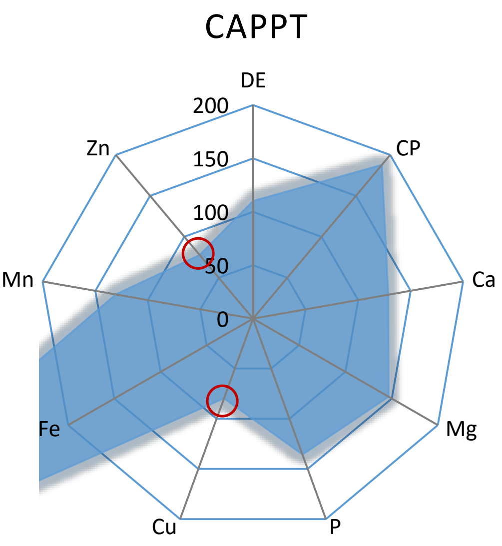


Figure 5. 8 Radar charts showing percentage of requirements for a 500 kg sport horse in moderate work fed 5 kg of working feed and 2.5 kg each of teff and lucerne

The cool group of rations are represented by EQUCNP15 and VITC feeds (Figure 5.9). VITC shows large overprovision with CP, Ca, Mg, Cu and Mn at close to 200% of requirements while P and Zn are close to 150% of requirements and Fe is again exorbitantly oversupplied. In contrast the EQUCNP15 ration shows lower percentages of oversupply of Ca and Mg between 100 - 150% with DE, P, Cu, and Zn close to 100% of requirements and CP at 200% of requirement and once again Fe is oversupplied. It is evident that high concentrate feed intake is not correcting the mineral under provision. Once mineral provision is optimised, feeding levels of concentrate diets can be adjusted.

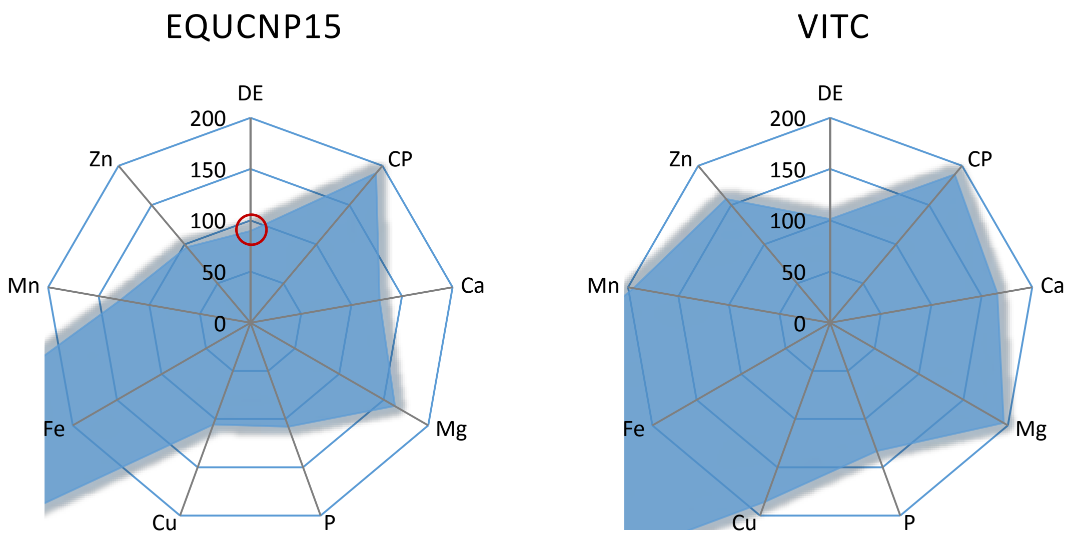


Figure 5. 9 Percentage of requirements supplied by rations of 5 kg of cool feeds and 2.5 kg of teff and 2.5 kg of lucerne for a 500 kg horse in moderate work

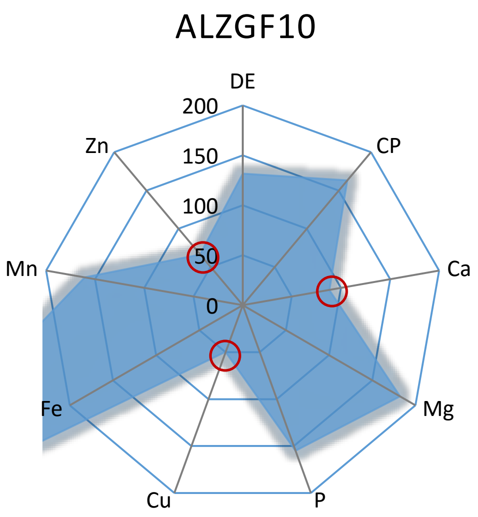
d) Adult Arab hack weighing 400 kg, eating 2% of BW

A 400 kg Arab used for hacking, falls within the light work category and eats a fodder: concentrate ratio of 80:20. The ration consists of 8 kg of mixed hay and 2 kg of feed from the leisure group. The nutrient content of the rations are shown in (Table 5.8) where it can be seen DE, CP, Mg, P, Mn and Fe are oversupplied and Cu and Zn are underprovided. Mineral levels differ with the feeds used. Cu and Zn are below the 120 mg and 480 mg recommendations in all the feeds. ALGF10 and ROMH do not meet Ca requirement.

Table 5. 8 Horse requirements and ration nutrient provision for a 400 kg Arab hack eating 2 kg of feed and 8 kg of mixed hay (100%DM)

	DE MJ	CP G	CA G	MG G	P G	CU MG	FE MG	MN MG	ZN MG
REQUIREMENTS	**67**	**559**	**36**	**11.4**	**21.6**	**120**	**320**	**480**	**480**
LEISURE GROUP MEAN	88	838	42	18	26	89	1826	776	386
ALGF10	88	913	32	21	33	60	1537	771	319
VUV10	85	785	41	17	24	94	1620	856	464
ROMH	86	803	34	17	25	79	1605	793	385
EQUT&L	86	869	47	19	26	88	2189	800	369
EQFMF12	87	778	50	17	23	78	1588	758	324

Graphically represented (Figure 5.10), the ALGF10 and VUMV10 feeds demonstrate several shortcomings. DE, CP, Mg, Fe and Mn are oversupplied and a slight undersupply of Ca occurs in the ALZGF10 ration and underprovision of Cu and Zn occurs. The VUMV10 shows more uniform mineral provision with only Cu undersupplied and Fe is again vastly oversupplied.



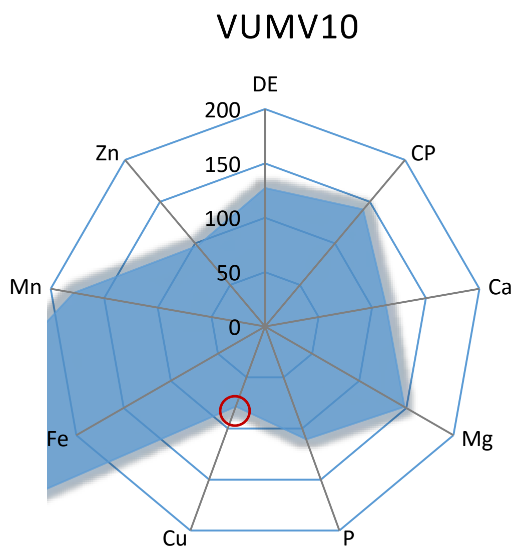
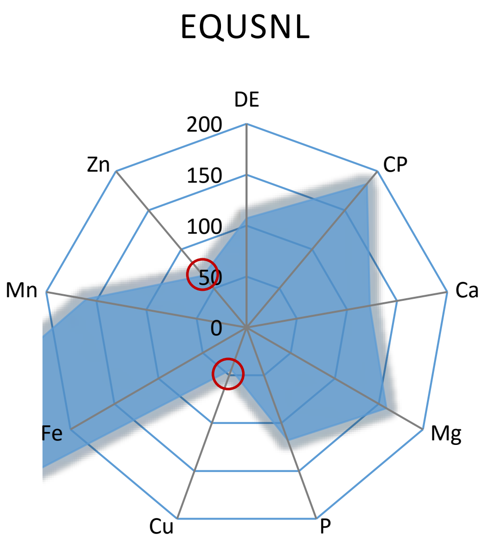


Figure 5. 10 Percentage of requirements for a 400 kg Arab hack provided by 2 leisure feeds fed at 2 kg with 8 kg of mixed hay

e) Aged, overweight pony with Equine Metabolic Syndrome (EMS), weighing 200 kg , eating 1.75% of BW

The last pony is a 200 kg overweight, aged pony with EMS grazing sweetveld and being fed 500 g of either a veteran feed (VITVET) or one designed for laminitic and metabolic horses (EQUSNL) as per feeding recommendations. The feed intake was given at 1.75% of BW in an attempt to restrict calory intake in this pony. The two radar charts (Figure 5.11) have very similar shapes showing these two feeds supply similar nutrient levels. It can be seen that the feeds supply DE slightly higher than requirements with a large oversupply of CP in both rations and this is in an overweight pony on feed restriction. The EQSNL provides 50% of the Cu requirement and VITVET aproximately 75% of Cu requirement and both rations are slightly deficient in Zn. Fe and Mn are oversupplied in both rations. It would be possible to trim DE and starch provision in this overweight, EMS pony if mineral levels were matched to requirement.



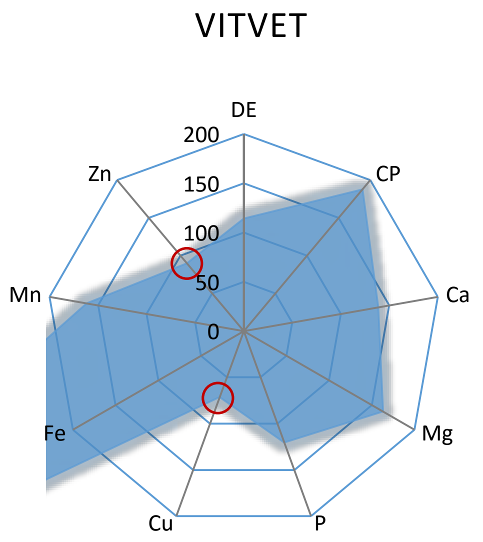


Figure 5. 11 Radar charts showing nutrient provision as a percentage of requirements for a geriatric pony fed 500 g of feed designed for aged horses or horses with EMS, and grazing sweetveld.

5.4 Discussion

Siciliano (2015) reports a significant proportion of a horse's daily nutrient requirements can be obtained from pasture which can be seen in this study where kikuyu provides sufficient DE, CP and certain of the minerals for the majority of horse classes. Hussein and Vogedes (2003) investigated the ability of various USA forages to provide horses with their required nutrient requirements and report high-quality forages are capable of maintaining idle horses and those in light work while growing horses, mares in late pregnancy and lactation and horses in heavy work often need supplementary feeding. A similar situation is evident in this study where kikuyu and lucerne provide sufficient DE for adult horses but growing and lactating mares would need supplementary grains to provide extra energy and only idle horses would receive their daily DE requirement from the other fodder types. CP in lucerne and kikuyu is sufficient for all classes of horses but may require individual amino acid supplementation for growth and lactation depending on the age and fibre content of the plants. Sweetveld provides adequate CP for adult horses including those in hard work but the fibre content and age of the grass will have a major role in the digestibility of the protein and supplementation may be necessary in working and breeding horses. Sourveld has insufficient CP content for any of the horse classes while eragrostis, mixed hay and teff provide adequate CP for idle and light work but once again the age and fibre content will affect availability of the protein and supplementary CP sources may be needed.

Siciliano (2015) reports the mineral content of forages varies widely although some generalisations can be made - grasses contain less Ca than legumes and fodder levels of P and Cu, Zn are generally low. Mn content varies from deficient to adequate and Fe content is always high. In this study, similar trends are seen with lucerne which contains significantly more Ca than the grasses. Saastamoinen *et al.* (2012) report the P and trace mineral content of fodder to be lower than levels reported in feed tables and this is found in this study where the P, Cu and Zn content of the fodder is low. P content in eragrostis, mixed hay and teff is only adequate for idle horses while sweetveld and teff provide sufficient P for horses in moderate work but not breeding or growing horses. Veld has low Ca content which will only maintain idle horses while P content is insufficient for all classes of horse. Eragrostis, sweetveld and teff have an ideal Ca:P ratio of between 1.2:1 and 2:1 whereas other groups have higher Ca:P ratios ameliorated in kikuyu where the bulk of Ca is unavailable. Mg contents are sufficient in all fodder for all classes of horses.

As found by Siciliano(2015), Fe and Mn content exceeds requirement for all horse classes in all the fodder groups besides lucerne where Mn content is insufficient to meet the needs of horses in heavy work and lactation. Teff is the only fodder to provide sufficient Cu for idle horses and yearlings but not working, breeding or growing animals older than 12 months. Zn content is insufficient in all fodder groups besides teff where the use of Zn in fertiliser in the production of the hay will raise Zn content. Reduced absorption of Zn is likely to occur due to high Fe content of fodder and feeds formulated to balance rations need to take the ratios between all trace minerals into account to reduce competitive absorption and optimise uptake of them all.

As found by Young (2011), CP is oversupplied in most horse rations currently used in SA. Mineral provision is erratic and unpredictable with large variations in between the feeds studied. The only certainty is the overprovision of Fe and slight oversupply of Mn. The other minerals exhibit a range of supply from being deficient to being over-supplied depending on the feed used in the rations.

Buchholz-Bryant *et al.* (2001) fed horses of different ages two rations constituted of 60% concentrate with 40% bermuda grass hay with either 133% or 275% NRC (1989) of Ca and P requirements, a level they found to occur commonly in commercial rations. They concluded young horses benefit more from extra Ca and P than mature or aged ones although all horses responded positively to the extra minerals. In the race and stud rations in this study where one would expect to find extra Ca and P for growth, one of each of the selected rations supplied extra Ca and P and the others were deficient in Ca and only just met P requirements in addition the Ca:P ratios were lower than the minimum of 1.2:1 recommended for Ca:P or inverted.

The rations examined in this study very seldom show uniform provision of both Ca and P but either supply high Ca and low P levels or in certain cases high P and low Ca. Absolute amounts of these minerals can be high, but it is most important that the ratio of Ca:P be above 1.2:1.

Caple (1982) found Ca:P imbalances occurred in Australian racehorses and suggested the resulting Nutritional Secondary Hyperparathyroidism (NSP), found in 40% of the test population, could be a major cause of lameness and fractures in racehorses. With the findings of Olivier *et.al* (1997) where 72% of lost training days resulted from lameness, the racehorse population in SA could be at serious risk of developing NSP resulting in lameness and devastating fractures due to poor Ca nutrition and the failure of the companies to provide adequate Ca in the feed.

Hintz (1973) reported decreased Mg absorption from unbalanced Ca:Mg or Mg:P ratios and reported decreased Mg absorption when a ration had a Ca:Mg ratio of 3.9:1. Although Mg provision appears to be higher than requirements across all the rations, the ratio between Ca and Mg in NRC (2007) requirements ranges from 2.4 to 5.3:1 and is far greater than the 2:1

upper level recommended by Johnson (2004) and Kellon (2008a). Relative to Ca the Mg content of the rations may be insufficient and may result in a relative deficiency of Mg which could explain certain cases of hyperactivity reported in many horses in the country, supported by the popularity of calming feeds and supplements on the market.

Pitzen (2006) found Fe provision in rations for cattle and horses were very high and Ramey (2011) found Cu and Zn provision in horse rations is typically low. A similar situation is found in this study. Both absolute deficiencies of Cu and Zn occur and relative to Fe, the provision of Cu and Zn is deficient in all feeds and fodder. The recommended ratio of 10:1 for Fe:Cu given by Johnson (2004) and Kellon (2008a) is not maintained in the horse rations and could cause secondary Cu and Zn deficiencies to occur. This is of even greater concern in horses with EMS as Nielsen (2012) and Pitzen (2006) have shown high Fe intake to be risk factors for the development of insulin resistance and laminitis, two of the symptoms found in EMS. Rations for aged and EMS horses showed excessive Fe content and are deficient in Cu and Zn which are needed to counter the oxidative damage from excess Fe.

The important consideration is that, for all the preceding scenarios, meeting mineral specifications means that concentrate provision (with all its concomitant issues in terms of energy, starch and CP overprovision) can be reduced. The horse can receive more fodder and return to a state more akin to its hindgut fermenter heritage.

5.5 Conclusions

Overall, feeds available to the South African horse owner do not complement the fodder types available in the country and mineral imbalances are evident in all the permutations of the various rations studied. Because of these discrepancies between nutrient provision and horse requirements, the horse owner would be better advised to spend their money on the brands of feeds which supply better mineral content and forget about the life-stage or work class their horse belongs to. It is evident from Chapter 4 that the feeds are all very similar in terms of macro-nutrients. Mineral profiles reflect factories rather than work classes.

It was hypothesised that current concentrate feeding regimes don’t complement SA fodder types. The fodder section implies that nutrient provision for idle, light and moderate work horses could possibly be supported by good quality kikuyu and lucerne, but, that as work rate increases concentrate feeds need to be supplemented.

Concentrate feeds don’t however demonstrate any cognisance of nutrient content of fodder types. In addition, no cognisance is taken of mineral contamination in raw materials or mineral sources in formulations. Basically a retrospective evaluation of feeds and fodder in the several classes of horse types reveals that there is no concerted feeding strategy for the meeting of horse nutrient requirements.

In the Final Overview and Future Research directives chapter that follows, recommendations for a unified equine feed formulation strategy are discussed.

Retrospectively, evaluations such as these prove that projected rations or formulations are falling short of intentions that other companion and production animal species have managed to model already. Expecting optimal performance in sport and recreational horses on “guesswork” and exaggerated feed intakes is a shocking disappointment to any well-meaning, registered, Animal Scientist.

Overview and future research directives

In SA, horses are fed a large proportion of their ration in concentrate feed with the addition of various fodder types to add bulk to the ration with little attention being paid to the nutrient provision from these fodder. Vast differences in horse management systems occur from rural and farm horses which eat 100% of their ration in fodder through to racing Thoroughbreds who are fed 80% of their ration in concentrate feed. High value sport and racehorses seldom get the opportunity to graze and are kept in stables for large parts of the day with limited turn out opportunities if any. These horses receive the bulk of their rations from concentrate feeds with limited fodder provision in the form of hay. Concentrate inclusions in rations range from 20 - 80 % depending on the management system in which the horse is kept.

These ratios of feed to fodder reflect the poor feeding strategies that are employed in the horse industry. They are disrespectful of the horse as a hindgut fermenter with a high requirement for fibre in the diet. Looking at ruminant nutrition it can be seen that feeding recommendations for concentrate feed are approximately 50% of the ration in a high producing dairy cow. In contrast, recommendations for horses, from the same feed company, are to feed up to 90 % of intake in starch rich concentrate feed! There are numerous examples of low feed intake supplements and licks in the beef and sheep industries that provide balanced nutrients for fodder based diets but nothing like this exists in the horse feed industry. Horses are overfed with poorly formulated, sugar–rich feeds and the resulting health and behavioural issues are ignored from a nutritional standpoint, a practice which is actively encouraged by the feed manufacturers themselves.

Coenen *et al.* (2011) have given new recommendations for fodder and starch inclusions in horse rations that introduce a novel perspective to horse nutrition and honour the horse as a hindgut fermenter with low starch and high fibre provision. They describe a model for ME calculations taking into account renal and methane energy expenditure of feedstuffs and mention that the model can be adapted as more information is obtained on different feedstuffs. Their fodder recommendation of 20 g/kg BW is far in excess of current feeding practices and the maximum starch recommendation of 1.1 g/kg BW per meal restricts the meal size of the current concentrate feeds to under 300 g/kg BW or 1.5 kg of concentrate feed per meal.

Using current horse requirements combined with the new starch and fibre recommendations and breed specific maintenance energy recommendations, with new research information coming in on metabolic models in horses, there is a solid platform for a definition of intake requirements in horses as per Emmans and Kyriazakis (2001). This will provide a good definition of updated requirements for horses as a base for future feed formulation. Using the fodder norms from Chapter 3 to provide a set of nutrient intakes, to provide a starting point to develop new feed formulation strategies which respect the physiology of the horse as a hindgut fermenter.

Our job as nutritionists would therefore be to formulate a ration that complements the nutrient and minerals provided in the fodder. Hence rations need not be so complicated and in fact the horse would benefit greatly in terms of current starch and CP overprovision in the current rations. Balanced mineral intakes would greatly enhance the health and wellbeing of horses via strengthened immune and skeletal systems and increased exercise tolerance from provision of anti-oxidant minerals in the diet.

So in the future, attention needs to be paid to

- Development of an accurate metabolic horse model
- Feed intake modelling
- Reformulation of the vitamin and mineral packs used in feed production
- Cognisance of mineral provision from raw products such as Fe contamination in di-calcium phosphate and magnesium oxide
- Use of the TMR approach to ration formulation with large fodder provisions

The formulation of a rational feeding model relies on:

1. Metabolic model taking all animal and environmental factors into account
2. Feeds database containing up to date, accurate, geographically specific information

3. Analytical services
4. On farm management and facilities

All of these are available and feed manufacturers could utilise these to bring a new paradigm into horse nutrition where horses are fed, as they were designed to be, with fodder based diets topped up with high quality fodder specific feeds, as is currently done in other production animal systems. In the future provision of nutrients to horses must combine all these attributes in the development of a rational feeding model for horses which can be adapted to formalise the approach to horse feeding in SA to make healthy, happy horses which are capable of performing the tasks required of them.

The aim of this study was to evaluate fodder and feeds which was done by means of chemical and statistical analysis to provide nutrient norms for fodder in SA.

In Chapter 3, PCA's were employed to form fodder groups of similar nutritional value to allow for nutritional profiles to be established for the different fodder types. Mineral levels in fodder varied between species and generalised fodder norms were established for seven fodder types. Kikuyu and lucerne formed two disparate groups containing high protein and mineral content compared to the other fodder which showed more uniformity in nutrient content and could be considered as one group for feed formulation purposes.

In Chapter 4, horse feed samples were analysed for their nutrient content and subjected to a PCA in an effort to group feeds of similar nutrient content. Balancer and fibre feeds were segregated from the rest of the feeds. The balance of the feeds could not be separated from one another on the basis of their proximate analysis. As feeds are sold to meet the needs of specific horse classes, each with varying protein and mineral requirements, this inability to segregate them into groups raises questions about feed formulation goals and the accuracy of horse feed production in SA. Feed nutrient content was compared to factory and label specifications to investigate the accuracy of information provided by companies. Poor correlations between actual nutrient content and specifications were found in certain brands of feed and mineral levels showed certain imbalances which could negatively impact horse health and performance.

Once generalised norms were established for fodder and feeds, these figures were used in Chapter 5 in a retrospective fodder and feed evaluation. Three main fodder types (kikuyu, lucerne and grasses) were evaluated and their potential to support varying work and life stage categories of horses was investigated. Grasses could only provide sufficient DE and CP for horses in the idle and light work category while kikuyu and lucerne could support horses up to moderate work level. Mineral provision in all the fodder types differed with Ca being deficient in kikuyu but excessive in lucerne. P was adequate in kikuyu and relative to Ca, deficient in lucerne, while grasses provided more balanced macro mineral intakes. In terms of trace minerals, horses were deficient in Cu and Zn across all the fodder while Fe was excessive for all horses. Mn content of grasses and kikuyu were sufficient for horses while lucerne required supplementation.

In the second section, hypothetical rations were created for model horses to mimic feeding routines and quantities used in the SA horse industry. Radar charts were used to show the nutrient provision of rations as a percentage of horse requirements to investigate absolute mineral provision and balance between minerals. While most diets met DE and CP requirements for the horses, it was evident that feeds are failing to provide horses with balanced mineral intakes. Certain feeds supplied more P than Ca and could result in the development of nutritional secondary hyperparathyroidism while trace mineral provision in the majority of the investigated diets showed excessive Fe and Mn provision with Cu and Zn deficiencies. Imbalances in the mineral provision could result in relative deficiencies of minerals and these imbalances and their effect on horses need further investigation. It is evident that brand selection has a bigger impact on horse nutrition than life stage or work category of the feed.

In conclusion it is evident there are currently large imbalances in the nutrient provision to SA horses and feed manufacturers are failing to carry out the mandate of providing balanced health-promoting nutrition to the detriment of the horse and their owners alike. New feed formulation goals and strategies need to be devised and implemented from the company level and be carried down to the horse owning public in the interests of improving health, performance and wellbeing of the horse.

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