

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**Digestibility of FiberEzy<sup>®</sup> and Timothy Haylage and Behavioural  
Observations and Voluntary Feed Intake of FiberEzy<sup>®</sup> and Rye  
Clover hay in Thoroughbred horses**

A thesis presented in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Massey University

Palmerston North, New Zealand

Deepa Patel

2014



## ABSTRACT

This thesis describes a field study, a laboratory study and two animal studies evaluating the production of timothy grass grown under New Zealand conditions, the composition of a commercially prepared ensiled lucerne and timothy mix ( FiberEzy®: a 50:50 timothy lucerne mix) and ensiled timothy grass;, the stability of FiberEzy® during 12 months of storage; and also the digestibility and voluntary feed intake of FiberEzy®, ensiled timothy grass and Rye-clover hay when fed to Thoroughbred horses.

For the field study, grass samples were collected from a representative section (6m x 16m) of a field in Reporoa, (Waikato, New Zealand) sown with timothy grass (*Phleum pratense L.*) on the 10<sup>th</sup> of March 2010 at Longitude 176°34'E, Latitude 38°39'S. The samples were collected at regular intervals to investigate changes in the dry matter (DM), acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin content of the crop over the growing season. There was an increase (17.7to 35.89%) in the dry matter content and lignin content (3.0to 6.3%) of timothy grass over time as the grass matured.

Once harvested and ensiled, a sample of timothy grass and FiberEzy® were analysed for total DM, percentages of: crude protein; crude fat; crude fibre; ash; gross energy; hot water soluble carbohydrates; pectin, (NDF), (ADF), lignin, and vitamin E content. Lab analysis showed that FiberEzy® had higher levels of crude protein, pectin, lignin, ash and vitamin E ( $p < 0.05$ ), and lower levels of crude fibre and NDF ( $P < 0.05$ ) than the timothy grass.

A shelf life stability study of FiberEzy® (was carried out by sub-sampling a stored bag of the product every 3 months and analysing for total DM, percentage crude protein, percentage hot water soluble carbohydrates and vitamin E (mg/kg) for a total of 12 months. During the storage trial, analysis of FiberEzy® showed an increase in the content of vitamin E (37.6to 124.9mg/kg) over time.

A digestibility study was conducted to compare FiberEzy® and New Zealand grown and ensiled timothy haylage on Thoroughbred horses. FiberEzy® and timothy haylage was offered on a DM basis at 2.5% of the body weight to four Thoroughbred horses ( $10 \pm 1$  yr;  $562.5 \pm 30.7$  kg initial BW). The horses were stalled individually in loose boxes (4 x 4

m) lined with rubber matting. Horses were randomly paired and assigned to be fed FiberEzy® or timothy haylage for two 18 day periods, in a 2 x 2 Latin square experimental design. Each 18 day period comprised of a 14 day dietary adaptation period where the horses were habituated to the pens and diet, and a 4 day (96 h) total faecal collection. The apparent digestibility of DM and GE were measured on days 15-18; at 12 hourly intervals faecal material from each horse was collected, weighed, and a subsample was taken and frozen at -20°C. One kg samples of the feed were collected and frozen at -20°C on day 15. The energy content of the FiberEzy® and timothy haylage varied between the first and second part of the study, with the DE of timothy haylage varying from 6.9 MJ/kg DM to 9.4 MJ/kg DM. Total energy intake of FiberEzy® and timothy haylage varied between horses, and between groups, with horses fed timothy haylage after FiberEzy® reducing their total energy intake. The apparent digestibility of FiberEzy® tended to be greater than that of timothy haylage. The apparent DM, crude protein and energy digestibility of the feed was similar to values reported in other studies using young horses.

A voluntary feed intake (VFI) trial was conducted using six thoroughbred geldings, ( $10 \pm 1$  yr;  $550.6 \pm 15.8$  kg) were offered FiberEzy® or Rye-clover hay at >3% of the body weight. The horses were again stalled individually in loose boxes (4 x 4 m) lined with rubber matting. Voluntary feed intake was measured over 17 days. Day 1 to 8 was the adaptation phase and from day 9-17 of the study the horses were scan sampled and videoed for two hours every morning (9:30-11:30 am) after feeding and two hours in the evening (4:30-6:30 pm) after feeding. There were significant differences in VFI between time periods ( $24.3 \pm 0.9$  vs  $17.8 \pm 0.4$  kg DM /day,  $p < 0.05$ ) and between feeds (FiberEzy®:  $24.3 \pm 0.4$  vs. Rye-clover hay:  $17.8 \pm 0.7$  kg DM /day) but not an interaction. Behaviours were typical of loose box housed horses and differences in feeding behaviour observed correlated with the differences in VFI measured between feeds.

The results of this thesis suggest that FiberEzy® is a suitable alternative to concentrate-based supplementary feed

## **ACKNOWLEDGEMENTS**

This research project would not have been possible without the support of many people. I would like to offer my sincere thanks to my supervisors Dr David Thomas, Dr Erica Gee and Dr Chris Rogers for their time, support and direction.

I am grateful to the Ministry of Science and Innovation for their assistance in funding this project, and to Fiber Fresh Feeds Ltd for providing me with the opportunity to do this project.

I would also like to thank the LWT Animal Nutrition Ltd team for the use of their facilities and assistance during the voluntary feed intake and digestibility study

This project would not have been successful without help from Sara Azarpeykan, Asmad Kari, Folusho Doris Adeyinka, Sharini Somasiri, Lana Bishop and Karlette Fernades.

I wish to express my appreciation and gratitude to my husband Tejas Patel and my Dad, family and friends for their continued support and encouragement.

Thank you.

## TABLE OF CONTENTS

### DIGESTIBILITY OF FIBEREZY® AND TIMOTHY HAYLAGE AND BEHAVIOURAL OBSERVATIONS AND VOLUNTARY FEED INTAKE OF FIBEREZY® AND RYE CLOVER HAY IN THOROUGHBRED HORSES

ABSTRACT .....	3
ACKNOWLEDGEMENTS .....	5
LIST OF FIGURES .....	8
LIST OF TABLES .....	9
LIST OF ABBREVIATIONS.....	11
INTRODUCTION .....	12
AIM OF THE STUDY.....	13
THESIS OUTLINE.....	13
<b>CHAPTER ONE</b> REVIEW OF THE LITERATURE.....	15
INTRODUCTION .....	15
1.1 The Digestive System of the Horse.....	15
1.1.1 The Mouth: .....	16
1.1.2 The Oesophagus: .....	17
1.1.3 The Stomach: .....	17
1.1.4 The Small intestine: .....	18
1.1.5 The Hindgut: .....	19
1.1.6 Accessory Digestive organs:.....	20
1.1.6.1 Liver: .....	20
1.1.6.2 Pancreas:.....	20
1.2 Forages for domesticated horses .....	21
1.2.1 Conserved Forages:.....	21

1.2.2 Nutritional component of ensiled forages .....	25
1.2.3 Various diseases associated with Haylage.....	26
1.3 Timothy as a Feed for Horses.....	28
1.4 Lucerne as a feed for horses .....	30
1.5 Apparent Nutrient Digestibility.....	32
1.6 Voluntary Feed Intake .....	33
1.7 Feeding and various different behaviours in Horses .....	36
1.7.1 Ingestive Behaviour .....	36
1.7.2 Abnormal Feeding Behaviour .....	39
1.7.3 Stereotypic Behaviour .....	39
1.7.4 Agonistic Behaviour .....	40
1.7.5 Communicative Behaviour.....	40
1.8 How diets and various factors can affect the behaviour .....	41
1.9 Behavioural Measurement.....	43
1.9.1 Ethograms.....	43
1.9.2 Scan Sampling .....	44
1.10 AIM OF THE THESIS .....	46
1.11 HYPOTHESES.....	46
1.12 REFERENCES .....	47
<b>CHAPTER TWO</b> Field sampling of Timothy, Lab analysis of FiberEzy® and ensiled Timothy and a shelf storage trial of FiberEzy®. ....	50
2.1 Abstract .....	50
2.2 Introduction .....	52
2.3 Methods and materials .....	54
2.3.1 Sample collection of Timothy .....	54
2.3.2 Analysis of ensiled FiberEzy® and Timothy.....	56

2.3.2 Shelf storage of FiberEzy® .....	57
2.4 Results .....	58
2.4.1 Field sampling of Timothy.....	58
2.4.2 Nutrient composition of FiberEzy® and Timothy .....	58
2.4.3 Storage trial of FiberEzy® .....	59
2.5 Discussion.....	61
2.5.1 Field sampling of Timothy.....	61
2.5.2 Storage trial.....	61
2.6 References.....	63
<b>CHAPTER THREE</b> Apparent digestibility of FiberEzy® and Timothy ( <i>Phleum pratense</i> ) haylage in Thoroughbred horses. ....	65
3.1 Abstract .....	65
3.2 Introduction .....	66
3.3 Methods and materials .....	68
3.3.1 Animals .....	68
3.3.2 Diet.....	69
3.3.3 Environment .....	69
3.3.4 Experimental design .....	69
3.3.5 Collection of samples.....	69
3.3.6 Chemical analysis.....	70
3.4 Results .....	70
3.5 Discussion.....	72
3.6 References.....	74
<b>CHAPTER FOUR</b> Voluntary feed intake and Behavioural observation of Thoroughbred horses fed FiberEzy® and Rye Clover hay .....	76
4.1 Abstract .....	76
4.2 Introduction .....	77

4.3 Methods and materials .....	78
4.3.1 Animals .....	78
4.3.2 Diet.....	78
4.3.3 Environment .....	78
4.3.4 Experimental design .....	79
4.3.5 Behavioural measurement .....	80
4.3.6 Behavioural analyses .....	82
4.4 Results .....	82
4.4.1 Behaviour Activity.....	83
4.5 Discussion.....	86
4.6 References.....	87
<b>CHAPTER FIVE</b> General Discussion.....	89

## **LIST OF FIGURES**

Figure 1.1: Digestive system of the horse.....	16
Figure 1.2: Timothy Grass .....	27
Figure 1.3: Lucerne in the field .....	29
Figure 2.1: Timothy grass within the sample area (a) 15 days before harvest, (b) at harvest, (c) 15 days post-harvest and (d) 45 days post-harvest.....	54
Figure 2.2: The areas where the sample was collected from (a) 15 days pre-harvest, (b) at harvest, (c) 15 days post- harvest and (d) 45 days post-harvest.....	55
Figure 2.3: Mould growth on bags of FiberEzy® during storage at room temperature in the Nutrition Laboratory (Massey University, Palmerston North). .....	60
Figure 4.1: Average voluntary DMI (kg/d) by Thoroughbred geldings fed on FiberEzy® (FE; n =6) or Rye Clover hay (RCH; n=6). .....	82
Figure 4.2: A representation of the behaviour of a single horse on two different feeds.....	83
Figure 4.3: A collective behaviour shown by the two group of horses on two different feeds i.e. FiberEzy® and RCH during the first Voluntary Feed Intake period. ....	84

Figure 4.4: Collective behaviour shown by the two group of horses on two different feeds i.e. FiberEzy® and RCH during the second Voluntary Feed Intake period. ....85

## **LIST OF TABLES**

Table 1.1: Moisture content and dry mater content of different conserved forages .....	17
Table 1.2: Advantages and Disadvantages of haylage .....	21
Table 1.3: Apparent Dry Matter, Crude Protein and Crude Fibre percentages of Timothy ( <i>Phleum pratense</i> ) .....	25
Table 2.1: Analysis of field samples of timothy .....	58
Table 2.2: Nutrient composition of FiberEzy® and timothy.....	58
Table 2.3: Selected nutrient composition of FiberEzy® during a 12 month storage trial (on a DM basis).....	59
Table 3.1: Apparent digestibility of feed by each horse .....	71
Table 3.2: DE content DM (MJ/kg) for FiberEzy® and timothy .....	72
Table 4.1: Ethogram of Stereotypic Behaviour measured incorporated from Cooper et al. (2005) .....	80

## **LIST OF ABBREVIATIONS**

BCS	Body condition score
BW	Body weight
CP	Crude protein
DE	Digestible energy
DM	Dry matter
GE	Gross energy
NRC	National Research Council
VFI	Voluntary feed intake
FE	FiberEzy®
T	Timothy grass haylage

## INTRODUCTION

New Zealand has a reputation for breeding high quality, internationally competitive race horses (Fennessy, 2010). Breeding Thoroughbred horses is an important part of New Zealand's equine industry (Morel et al., 2007; Stowers et al., 2009), and the sale of yearling horses provides a large portion of revenue for breeders (Flores et al., 2011).

The New Zealand equine industry is largely pasture based due to the mild climate which allows pasture growth throughout the year (Pearce et al., 1998). Because of this horses are able to obtain a significant portion of their nutrient requirements from pasture (Hoskin and Gee, 2004). While foals can achieve acceptable growth rates when raised on good quality pasture alone (Grace et al., 2003; Brown Douglas et al., 2005), it is common practice to provide supplementary concentrate feed to growing Thoroughbred horses despite the availability of pasture (Stowers et al., 2009; Hirst, 2011). This frequently occurs when stud managers are unsure of the extent to which weanlings meet their nutrient requirements through pasture (Stowers et al., 2009; Hirst, 2011). Cereal grains are commonly added to feed rations to increase the dietary energy as most young horses require more energy and protein than forage can provide alone (Moore-Colyer and Longland, 2000; Connysson et al., 2006; Brown-Douglas, 2012). There is a growing interest in high-energy, fibre-based feeds as an alternative to providing dietary energy through grain (Murray et al., 2005). Energy dense fibrous feeds support the normal function of the hind gut and may reduce digestive problems (Miraglia et al., 2006; Willing et al., 2009), while the risk of developmental orthopaedic disorder may be decreased by feeding young horses low starch diets (Miraglia et al., 2006).

High fibre forage-based feeds such as lucerne (*Medicago sativa*), can reduce the risk of gastric ulceration (Nadeau et al., 2000; Lybbert et al., 2007), and promote natural feeding behaviours (Hill, 2007). Lucerne hay, also known as alfalfa hay, is a popular forage for horses (NRC, 1989; Small, 1996; Crozier et al., 1997), has a higher nutritive value than grasses (NRC, 1989) and is preferred over grass hays by horses (Cymbaluk, 1990; LaCasha et al., 1999; Rodiek and Jones, 2012).

Timothy grass (*Phleum pratense*) is a popular forage in North America. In the cold winters of North America, forage production depends on hardy species such as timothy grass which is grown mainly for silage and hay (Berg et al., 1996a). Timothy grass is not currently grown commercially in New Zealand, but may have potential as a nutritive feed for horses.

Forage can be conserved through an ensiling process where anaerobic fermentation of non-structural carbohydrates occurs (NRC, 2007). As ensiled forages are harvested at an earlier stage of maturity than hay (Peiretti and Bergero, 2004; Connysson et al., 2006; Ragnarsson and Lindberg, 2008), they have a higher nutritive value than corresponding hays (Hale and Moore-Colyer, 2001; Bergero and Peiretti, 2011)

### **Aim of the study**

The aim of this study was to determine the digestibility and voluntary feed intake of two conserved forages in stabled horses. The objectives of the project were; (i) to determine the digestibility of FibreEzy® (a haylage made with the 50:50 mixture of lucerne and timothy) and Timothy grass alone when fed to thoroughbred horses; (ii) to determine if there is a difference in the palatability of FibreEzy® haylage or timothy haylage by horses (iii) establish the Voluntary Feed Intake (VFI) of FibreEzy® versus Rye-clover hay; (iv) make behavioural observations of thoroughbred horses during the voluntary feed intake period when they are fed two conserved forages under the same stabled conditions and feeding environment.

### ***THESIS OUTLINE***

This thesis is presented as four chapters:

Chapter 1 reviews the relevant literature outlining the digestive system of the horse, the various feeds used for domesticated horses, the diseases associated with haylage and feeding and feeding behaviours in horses. The field sampling of Timothy and a shelf life study of FiberEzy® are discussed in Chapter 2. The apparent digestibility of FiberEzy® when fed to Thoroughbred horses is studied in Chapter 3. The minimum duration of total faecal collection that is required for accurate apparent digestibility

measures is also determined in this chapter. Determination of the voluntary feed intake of FibreEzy® versus Rye-clover hay and behavioural observation of thoroughbred horses is discussed in Chapter 4.

## **CHAPTER ONE**

### ***REVIEW OF THE LITERATURE***

#### **Introduction**

##### **1.1 The Digestive System of the Horse**

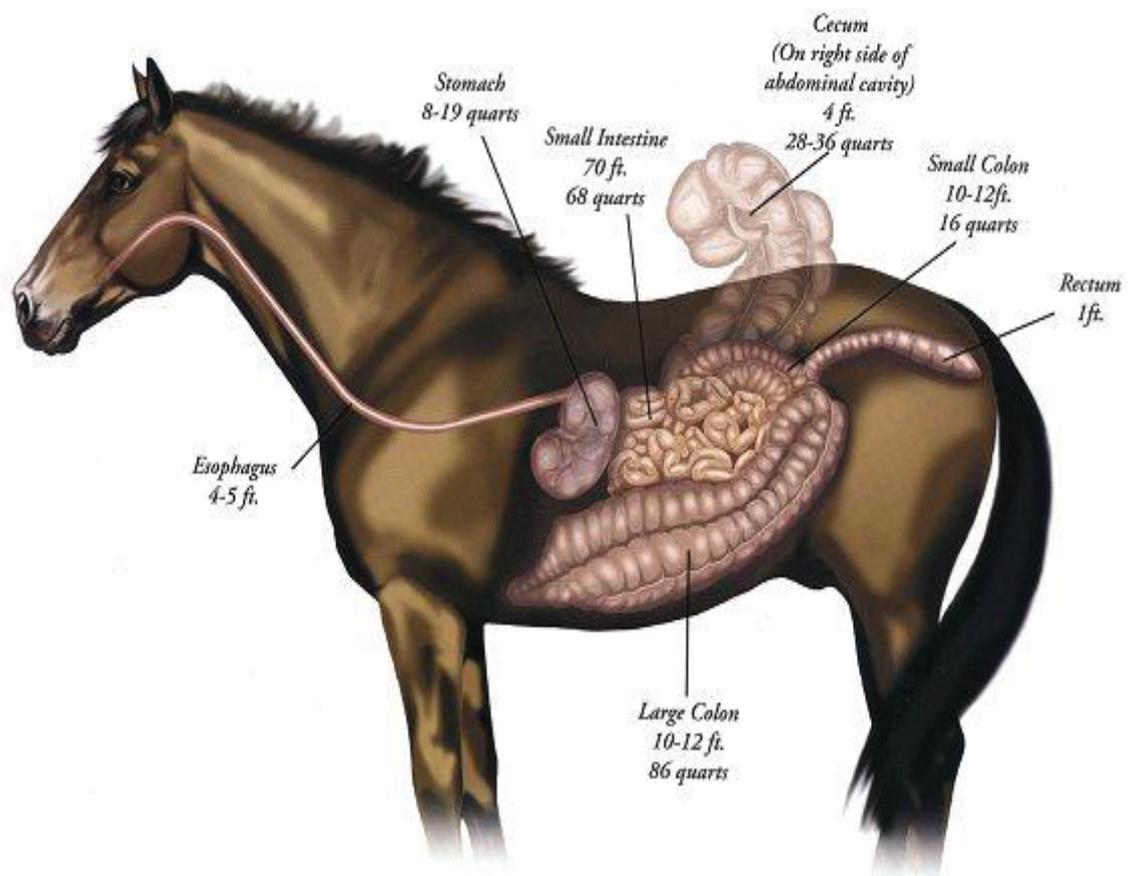
Horses are classified as simple stomached, monogastric non-ruminant herbivores (Hintz et al., 1994), or roughage eaters. They are grazing animals with digestive systems designed for constant consumption of plant food (Davis, 2009).

The digestive tract of the horse consists of the mouth, pharynx, oesophagus, stomach, small intestine (duodenum, jejunum, and ileum), large intestine (caecum, large colon, and small colon), rectum and anus. The liver and pancreas are associated with the digestive tract and release part of their secretions directly into it. The functions of the digestive tract include prehension, digestion, absorption and initial storage of nutrients (Evans, 2000).

The domesticated horse consumes a variety of feed from fresh forage with a high moisture content , to cereals with large amount of starch and hay in the form of physically long fibrous stems, to salt licks and water. In contrast the wild horse evolved and adapted to a grazing and browsing existence in which it selects succulent forages containing relatively large amounts of water, soluble proteins, lipids, sugars and structural carbohydrates, but little starch (Frape, 2004).

Today's horse is often confined to a stable for most of the day and fed large meals low in fibre (Hintz, et al., 1994). Domestication of the horse has led to changes in the feeding pattern by restricting the feeding time and introduction of feed materials such as starchy cereals, protein concentrates and dried forage, which may cause digestive and metabolic upsets. To prevent metabolic or digestive upsets, proper understanding of the normal form and function of the alimentary canal is fundamental when discussing feeding and nutrition of the horse. The digestive system of the horses is

explained below and the different parts of the digestive system are shown in Figure 1.1.



**Figure 1.1:** The digestive system of the horse  
(<http://www.admani.com/allianceequine/equinedigestion.htm>)

### 1.1.1 The Mouth:

The mouth is where the digestion begins. The lips, tongue and teeth of the horse are suited for prehension, ingestion and alteration of the physical form of the feed to that suitable for propulsion through the gastrointestinal tract in the state that facilitates admixture with digestive juices (Frape, 2004). The lips of the horse's mouth are very sensitive tactile organs, they are quite mobile and help in sucking of water and selection and prehension of food (Evans, 2000). The teeth act as a prehensile organ and harvest the feed, which means they bring the grass to the mouth (Davis, 2009). Horses chew many more times when eating forage compared to concentrate feed (Meyer *et al.*, 1975).

The physical presence of feed material in the mouth stimulates the secretion of a copious amount of saliva. The salivary glands play an important role in the digestion of feed and forage (Davis, 2009). The saliva produced not only moistens the food but assists in the movement of the food through the tract, and also as the beginning of carbohydrate digestion as it contains amylase i.e. a minor enzyme that helps digest carbohydrate (Evans, 2000). The mixture of food and saliva is known as chyme. The act of swallowing is known as deglutition. Deglutition occurs when the highly muscular tongue pushes the bolus of the food to the back of the mouth, towards the pharynx. The pharynx connects the mouth with the oesophagus and connects the nasal cavity with the larynx and trachea leading to the lungs (Davis, 2009; Evans, 2000). There are two cavities in the pharynx : the nasopharynx (part of the respiratory channel) and the oropharynx (part of the digestive tract) (Evans, 2000).

### **1.1.2 The Oesophagus:**

The oesophagus is a muscular tube which extends down the left side of the neck leading from the pharynx to the stomach which lies just caudal to the diaphragm (Evans, 2000; Frandson et al., 2009); By peristalsis (i.e. muscular contraction) the food moves down the oesophagus. The tunica muscularis of the oesophagus is responsible for peristalsis which pushes the bolus down the oesophagus into the stomach. The oesophagus joins the stomach at the cardia, a part so named because of its proximity to the heart (Evans, 2000; Frandson, et al., 2009).

### **1.1.3 The Stomach:**

The stomach is a 'J' shaped elastic organ (Frandson, et al., 2009), which is small in relation to the animal and to the volume of fodder consumed (Dyce et al., 2009). Its capacity is 5-15 litres, about 8-10% of the digestive tract capacity (Hintz, et al., 1994). Food enters the stomach from the oesophagus through a valve known as the cardiac sphincter. The valve stops the movement of the food back into the oesophagus and therefore the horse is unable to vomit or belch (Dyce, et al., 2009; Evans, 2000; Hintz, et al., 1994). Food leaves the stomach through another valve known as the pyloric sphincter and enters the duodenum, the first part of the small intestine.

The stomach consists of two limbs that meet at a ventral angle. The left limb comprises the fundus, usually large and often termed as *saccus cecus* (a blind sac) as well as the body; the right limb or pyloric section is much narrower and extends across the midline to join the duodenum (Dyce, et al., 2009). A stepped edge (*margo plicatus*) divides the interior between a large non-glandular region, occupying the fundus and part of the body, and a glandular region (Dyce, et al., 2009).

#### **1.1.4 The Small intestine:**

The length of the small intestine is relatively short i.e. 21-25m (Dyce, et al., 2009; Frape, 2004). The small intestine is composed of three parts that contains 30% of the digestive tract capacity: duodenum, jejunum and ileum (Evans, 2000; Hintz, et al., 1994). The pancreatic and bile duct enter the duodenum. Bile salts are secreted continuously in to the digestive tract because the horse has no gall bladder to store them and the bile is necessary to emulsify lipids (fats) (Evans, 2000).

Pancreatic juice contains enzymes for protein digestion and carbohydrate digestion. The small intestine is the primary site of protein digestion and absorption of amino acids. About 60-70 % of the protein eaten may be digested and absorbed before reaching the large intestine (Evans, 2000). Most soluble carbohydrates are digested in the small intestine. The end products are glucose and volatile fatty acids, which are absorbed and used for energy. The small intestine is also the primary site of fat digestion and absorption therefore; it is presumed that the fat soluble vitamins A, D, E and K are absorbed here, as are the B vitamins. Most calcium, zinc and magnesium are absorbed here and the same amount of phosphorus is absorbed here as in the large intestine (Evans, 2000).

Mesentery suspends the small intestine from the dorsal body wall and can be named according to the part of the intestine supported i.e., mesoduodenum, mesojejunum and mesoileum. The mesoduodenum is generally short, so the location of the duodenum is relatively fixed. The mesojejunum and mesoileum are collectively called

the greater mesentery and the length of the greater mesentery permits considerable motility of the intestinal mass (Frandsen, et al., 2009).

The small intestine of the horse is not unusual when compared with other domestic animals. However, its hindgut has several distinguishing characteristics. It is much larger than that of cattle, sheep, or swine and contributes more to the relative capacity of the equine tract (Hintz, et al., 1994).

#### **1.1.5 The Hindgut:**

The large intestine, composed of the caecum and the colon, has a large capacity. The caecum, a pouch close to the ileum, may hold as much as 70 litres but normally contains about 30 litres (Dyce, et al., 2009; Evans, 2000). The colon has sacculations with narrow and broad compartments demarcated by discrete bands of smooth muscle and connective tissue called teniae. The teniae are formed by longitudinal muscle and provide intestinal support but yield to intestinal distension caused by fermentation. The sacculations reduce the rate of digesta passage and thus enhance microbial fermentation and digestion because the digesta is exposed to the bacteria for a longer period of time (Hintz, et al., 1994). This arrangement likely evolved during the years when horses spent most of their time grazing. Therefore, the microbial population of the hindgut received a slow and steady supply of fermentable material (Hintz, et al., 1994).

The colon consists of the ascending, transverse and descending parts. The first two together constitute the “large colon” and the third constitutes the “small colon” The ascending, or greater, colon is composed of four parallel limbs separated by three flexures, the right ventral colon, sternal flexure, left ventral colon, pelvic flexure, left dorsal colon, diaphragmatic flexure, and right dorsal colon (Dyce, et al., 2009; Evans, 2000).

The large intestine serves as a fermentation vat (Evans, 2000; Frape, 2004). The microbial flora found are similar to those found in cattle. These bacteria break down

cellulose that reaches the large intestine into volatile fatty acids: acetic, propionic and butyric acids. The bacteria are able to use some non-protein nitrogen for protein synthesis, or at least increase nitrogen retention (Evans, 2000; Frape, 2004). Methane is a by-product of microbial fermentation. The caecum is the primary site of water absorption, but the large colon also plays an important role. B vitamins are synthesized and absorbed in the large intestine (Frape, 2004). The digestive tract ends in the rectum, which is 8-12 inches long, and the anus (Evans, 2000).

### **1.1.6 Accessory Digestive organs:**

#### **1.1.6.1 Liver:**

The liver is the largest gland in the body constituting 1-2% of the total adult equine body weight (Dyce, et al., 2009; Frandson, et al., 2009). The liver receives two blood supplies, the nutrient blood supply and the functional blood supply. The liver's digestive secretion, bile, leaves the liver through the hepatic ducts, which join the cystic duct from the gallbladder to form the common bile duct, which then passes to proximal duodenum into the lumen of which it opens in common with the pancreatic duct on the major duodenal papilla (Dyce, et al., 2009). All the domestic animals except horses have a gallbladder for storage of bile.

Liver cells are responsible for bile formation. Bile is a greenish-yellow salt solution consisting of bile salts, cholesterol, phospholipids (lecithin), and bile pigments (bilirubin). Hepatocytes synthesize the bile salts from cholesterol. Bile salts assist in digestion and absorption of lipids and the production and secretion of bile salts is the most important for the digestive function of the liver (Frandson, et al., 2009).

#### **1.1.6.2 Pancreas:**

The pancreas is a compound gland that has both endocrine and exocrine portions. The exocrine portion of the pancreas produces sodium bicarbonate and digestive enzymes, that break down protein, carbohydrates, and fats (Dyce, et al., 2009), which pass through the pancreatic duct to empty into the duodenum close to the opening of the bile duct (Frandson, et al., 2009).

The endocrine component comprises of pancreatic islets, which are clumps of cells that are scattered between the exocrine acini and are the source of insulin, glucagon, and gastrin; the islets are therefore of prime importance in carbohydrate metabolism (Dyce, et al., 2009; Frandson, et al., 2009).

## **1.2 Forages for domesticated horses**

The main component of any horse's diet should be forage (Burk et al., 2008). The horse is a social species, naturally living in herds and spending the majority of its time roaming and foraging in a diverse and seasonally-varying environment. As a non-ruminant herbivore it is well suited to a high fibre, low starch diet.

Domestication has resulted in a number of benefits to the horse, reflected in its continued prevalence and apparently increased life expectancy, but it has not been without its price. Especially in developed countries, horses kept for leisure purposes (which includes all competition and racing horses) are often confined, possibly away from conspecifics, within a stable for a large proportion of the day (Davidson et al., 2003). Due to increased energy requirements many horses now receive one to two large meals a day, consisting of feedstuffs with low water content and often a radically different nutritional profile from the high forage diet that they would be able or would choose to select in the wild. These modern practices have benefits but also potential disadvantages to the horse both nutritionally and behaviourally which may have an impact on welfare (Davidson, et al., 2003).

### **1.2.1 Conserved Forages:**

Forages may be conserved for later use when there is little fresh forage available or when animals are confined to stalls. Such conservation is achieved by drying, ensiling, or applying preservative (NRC, 2007). Forages preserved by ensiling are usually harvested at an earlier stage of maturity than forages preserved as hay. This results in greater digestibility of nutrients and a higher energy value of ensiled forages compared to those conserved as hay (Ragnarsson et al., 2008). Conserved forages can be chopped, ground and pelleted, cubed, or wafered after drying, whereas ensiled

forages are either bulk stored in bunkers or silos, or baled and wrapped in plastic film (NRC, 2007). The average DM content and water content of forages using the different conservation methods is shown in Table 1.1.

**Table 1.1:** Moisture content and dry matter content of different conserved forages

	Hay	Haylage	Silage
Water Content	28-34%	40-45%	65-70%
Dry Matter Content	66-72%	55-60%	30-35%

After (Frape, 2004; Lewis, 1995; Wilkinson, 2005).

### 1.2.1.1 Hay:

Stabled horses fed hay *ad libitum* spend almost as much time eating as free-ranging horses (Haupt et al., 2001). When fed hay *ad libitum*, horses in box stalls or tie (straight) stalls spend 60% of their time eating. Eating hay differs from grazing in several ways: the horse does not have to bite toprehend the forage; depending on the manner in which the hay is provided, the horse may have its head up, at chest height or down in the natural position. When given the opportunity, horses will remove the hay from the manger and place it on the floor (Sweeting et al., 1985), indicating their preference. Time spent eating hay increases when hay is available in several locations in the stack and when several types of hay are fed (Ninomiya et al., 2004).

The great difference between hay and natural forage is that hay is dry. The horse must obtain more fluids as water when eating hay rather than grazing. Some horses dunk their hay in their water, which may be an attempt to rehydrate it. The closer the hay is to the water source, the more likely the horse is to place the hay in the water (Bels, 2006).

Hay is the most traditional method of conservation of grass. As long as hay is composed of safe, nontoxic, nutritious plants, the stage of maturity of the crop at the time of cutting, the weather conditions and care to which hay making is subject are much more important characteristics than the species of plant present (Frape, 2004). Timothy hay is generally composed of more stalk and less leaf compared to meadow

hay and it also feels hard to touch. Hay tends to be less nutritious than haylage as it is cut later in the season (or at a more mature state. The quality and quantity of field dried hay for harvesting will depend on several factors such as, stage of maturity when cut, methods of handling the hay, moisture content present in the hay and weather conditions during drying (Frape, 2004).

In recent years, hay has largely been replaced by silage and haylage in equine diets (Müller & Udén, 2007).

#### **1.2.1.2 Silage:**

Silage consists of green forage preserved by fermentation in a silo for use as succulent fodder. The process of making silage is called ensiling. Silage is the product of controlled fermentation of green fodder retaining high moisture content (Frape, 2004). Big-bale silage, widely used in farming, and other plastic-wrapped silages have high DM content, minimal fermentation, no additives and a pH of 5-6. Plastic wrapped silage is subject to aerobic deterioration, owing to lower density than bunker silage and to the potential for punctures in plastic wrapping (Frape, 2004). Horses tend to use their sense of smell to select feeds therefore, it is important to ensure high quality preservation of silage so as to avoid production of any “off” aromas which would reduce intake that it is eaten in the required daily quantity (Wilkinson, 2005).

#### **1.2.1.3 Haylage:**

“Haylage” is the term often used to describe ensiled forages (Wilkinson, 2005). Haylage is essentially high dry matter (DM) silage, preserved in sealed silos, usually bales, with little fermentation because of the high DM of the crop at harvest, it is not hay because it has lower DM than traditional hay and cannot be stored like hay (Wilkinson, 2005).

Grass haylage is cut between early silage and hay stages and normally containing, after preservation, 55-70% DM. Haylage is being used increasingly in place of hay for feeding horses (Bergero et al., 2011; Frape, 2004), as it is dustless and a palatable feed with an enhanced nutritive value. Haylage is generally packed in large bales (Bergero et al., 2002). Ragnarsson and Linderberg (2008) reported that early cut timothy haylage

(stem elongation to flowering) has the necessary energy content to form the basis of diets for high performing horses. They also reported that late cut timothy haylage (seeding to late seeding) could be used to fulfil the energy and protein requirements for maintenance fed to horses.

Haylage is a well conserved feed compared to hay and therefore, the nutritive value is similar to that of the original grass, and because of the higher moisture content compared to hay, the risk of respiratory disease is less. Haylage can be packed according to its requirement in the stable, so it can be consumed in time without spoilage (Bergero et al., 2011).

To prepare haylage, stands of pure grass are cut at the bud stage and wilted for 48 h before baling tightly, and should not be fed to horses for at least a week after it is baled (Frape, 2004; Wilkinson, 2005) The haylage is baled at about 55-70% moisture and immediately wrapped with plastic, or placed in a bag, which reduces the presence of oxygen. After packing, the oxygen (O<sub>2</sub>) content drops almost immediately and a fall in pH soon follows (Jonsson, 1989). A pH lower than 4.6 inhibits growth of *Clostridium botulinum* (Dodds, 1994; Notermans et al., 1979), but several factors influence the acid tolerance of the bacteria, including strain, substrate, temperature, the presence of preservatives and water activity (a<sub>w</sub>). Fermentation then takes place and increasing acidity from the fermentation process drops the pH to about 4.5. At this pH, moulds and fungi cannot grow and at the end of the fermentation process the haylage will remain stable as long as the bag or wrapping remains fully intact (NRC, 2007). This process can take up to 8-10 weeks. Making good quality haylage requires farming expertise because if the grass is ensiled too dry or it doesn't contain enough soluble sugars, incomplete fermentation takes place. Good haylage has DM concentration between 500 and 700 g DM/kg fresh weight, (i.e. 55-70% DM) and the pH is between 4.5 and 5.5 (Davis, 2009; Wilkinson, 2005), the content of ammonia should be less than 5.0% and it should contain 7-12% of crude protein (Davis, 2009).

Initially when haylage was made for horses, the grass was cut earlier in the year when it was lush, higher in protein content and energy and lower in fibre. Whereas, today it

is cut later as the end product is more suitable. Hay has a lower water content compared to haylage which in turn contains less water than silage (Davis, 2009).

There is no clear definition of the difference between haylage and silage. In general, fermentation is restricted as dry matter content increases, this is reflected in higher pH and soluble carbohydrates and lower levels of fermentation acids (McDonald et al., 2002).

Table 1.2 describes some of the advantages and disadvantages of haylage.

**Table 1.2 : Advantages and Disadvantages of Haylage**

Advantages	Disadvantages
Quality of the cut forage is maintained	Improper preservation of haylage can cause botulism
Digestibility and palatability are not decreased with the use of preservatives	Handling problems may occur as haylage is twice the weight of dry hay ( due to 50% moisture)
Haylage has a higher protein and energy content compared to hay	Spoilage occurs if there is any sort of damage to the packing because of the entry of oxygen
Has a high moisture content and lack of dust	due to the risk of horses being exposed to botulism, they should be vaccinated prior to being fed haylage

Information taken from: (Battle et al., 1988; Lawrence et al., 1987; Wilkinson, 2005)

### **1.2.2 Nutritional component of ensiled forages**

The nutritional value of ensiled forages in horses has not been extensively investigated, historically it has been regarded as unsuitable to horses (NRC, 2007). However, the idea of feeding ensiled forages to horses is not new and feeding recommendations have existed, since before the middle of last century (Olsson et al., 1955). Anecdotal evidence suggests increased interest in feeding ensiled forages to horses (Ellis et al., 2005; Moore-Colyer et al., 2000; NRC, 2007).

When compared to hay, well preserved silage has less aeroallergens (Vandenput et al., 1997). In wilted silages clostridial and enterobacterial activities are normally minimal although some growth of lactic acid bacteria occurs (McDonald et al., 2002). By wilting the forage, the risk of adverse fermentation is reduced, as is the risk of botulism (NRC, 2007).

Moore-Colyer & Longland (2000) compared the intake of big-bale silage (DM 50%) and haylage (DM 68%) in horses and found that these products were consumed in larger amounts than hay and clamp silage (DM 34%). In contrast, a recent preference study on baled grass forages with different DM content showed that the product with the lowest DM content (31%) was preferred and consumed in larger amounts than hay and haylage (Müller & Udén, 2007).

The most successful producers and users of haylage seem to find that haylage with very high DM content is acceptable to most of the horses (Frape, 2004). Recently it has been shown that although simultaneously harvested, silage/haylage have higher digestibility in horses than hay (Muhonen et al., 2009), as haylage/ silage has a higher nutrient content compared to hay.

### **1.2.3 Various diseases associated with Haylage**

Ensiled forages are often used as horse feed, however the anaerobic condition of the ensiled forages favours the growth of *Clostridium botulinum*, the causal agent of botulism poisoning that can be fatal to horses (Ricketts et al., 1984). The endospores are more tolerant to heat, irradiation, oxygen and antibiotics compared to other bacteria (Lewis, 1995).

A higher DM content can also lead to a higher pH. Clostridial multiplication is inhibited below pH 4.6. It has been shown that proteolytic strains of *Clostridium botulinum* type B can produce toxins in grass silage (Notermans et al., 1979). Noncarrier-associated botulism poisoning (forage poisoning) of animals has been reported and type B has frequently been demonstrated in such outbreaks (Divers et al., 1986). Wilting may inhibit growth of *Clostridium botulinum* as the vegetative bacteria is quite sensitive to high osmotic pressure, but safe values are difficult to estimate (Hailu et al., 1991;

Jonsson, 1989). If the DM content of the silage is low (15% or below), the pH may fall in the beginning of the process increasing the possibilities of growth and toxin production (Dodds, 1994; Jonsson, 1989; Notermans et al., 1979). When used correctly, preservatives have been shown to improve feed quality.

Preservatives inhibit the growth of *Clostridium botulinum* by lowering pH and/or inhibition by nitrite (Dodds, 1994). If the silage is contaminated with animal carcass or manure preservatives have a limited ability to protect against botulism. It has also been shown that the effect of low pH and low  $a_w$ , in limiting toxin production is less, in a meat than in a grass medium (Notermans et al., 1979). Thus prevention of toxin production in contaminated silage may, therefore, prove difficult.

The hygienic quality of ensiled products for horses is a paramount, as horses do not possess the ability of ruminants to metabolize certain toxins. Care needs to be taken to ensure that no soil or animal carcass are ensiled with the forage as this can lead to proliferation of *Clostridium botulinum* (Ricketts et al., 1984). Haylage or silage can occasionally contain *Listeria spp* to which horses are highly susceptible (Ricketts et al., 1984). *Listeria monocytogenes*, the causal agent of listeriosis in horses, may also be found in poorly preserved haylage. Listeriosis has been reported in a number of Icelandic ponies that had consumed spoiled grass silage contaminated with the bacterium (Gudmundsdottir et al., 2004).

Fungal contamination of haylage or silage results in formation of mycotoxins (Dutton et al., 1984), such as dicoumarol which causes nasal bleeding, joint swelling, lameness, and respiratory difficulties (NRC, 2007). Mouldy hay or feed is known to cause colic symptoms in horses, and mould spores are known to cause heaves, a chronic obstructive pulmonary disease (Cuddeford, 1996).

Blister beetle toxicosis in horses occurs from ingestion of blister beetles present in lucerne hay cut in midsummer. Blister beetles contain a toxin called cantharidin, which causes blistering and bleeding of the intestinal tract with subsequent colic symptoms

and potential death, crimping the hay before baling kills and traps the toxic blister beetles in the hay (Lewis, 1995).

### 1.3 Timothy as a Feed for Horses

Timothy (*Phleum pratense* L.) is a tufted perennial grass; light grey-green, with rounded tillers, flat leaves and swollen bulb-like tiller bases. The foliage is dull and hairless with a short ligule but no auricle where the leaf blade joins the sheath. Its flower-head is a spike-like 'pipe-cleaner', which has prominent awn points (Shown in Figure 1). The inflorescence is a spike-like panicle, from which the grass obtained its old name of catstail (Langer, 1990) Seeds are small, rounded and pale. Timothy is a bunch-type, shallow-rooted, cool season grass. Its shallow root system, makes it unsuited to drought-prone soils (Hall et al., 1914), and it is sensitive to high temperatures.



**Figure 1.2 :** Timothy grass (<http://www.projectnoah.org>)

Timothy stores energy reserves for re-growth and tillering in its haplocorm or corm (enlarged bulbous structure) at the stem base. Its energy storage pattern makes it a better hay crop than a pasture species (Hall et al., 1914). Late to start growing, Timothy is not widely used in New Zealand, though it suits the Southern cool-temperate regions of the South Island, particularly moist heavy soils (AgResearch). Timothy is currently grown mainly on the deep, moist soils of Canterbury, Otago and Southland as crop and for seed production (Langer, 1990).

In cold-winter regions, forage production depends on hardy species such as Timothy which is grown mainly for silage and hay (Berg et al., 1996a). Because of the development of new grazing-type Timothy cultivars, it is also included in pasture mixtures (Kunelius et al., 2003). Timothy is most productive during spring and early summer under cool, long-day conditions with abundant moisture (Kunelius et al., 2006). Regrowth after defoliation is variable and usually slower than that of most other grass species. Growing Timothy in a mixture with other forage grasses and legumes tends to increase the productivity and reliability of forage crops that are under environmental stress (Berg et al., 1996b; Frame et al., 1987; Frame et al., 1985; Jørgensen et al., 1994).

Timothy is known to be a very palatable and nutritious grass (Langer, 1990). To thrive it needs medium to high fertility and adequate soil moisture. Because of these requirements and owing to its palatability and late-spring growth, Timothy does not successfully compete with ryegrass (Langer, 1990).

Timothy is grown primarily as hay for horses but is frequently included in pasture mixtures. It is less competitive in a legume mixture than most sod-forming grasses and is often grown in a legume mixture for hay (Hall et al., 1914). Timothy can be slow to establish and may fail when weed competition is severe during establishment. It establishes slowly, but once established it thrives on heavy, damp soils or under irrigation (Langer, 1990).

Oats are the most common companion crop, but early removal for silage or by grazing is necessary to reduce competition for light and moisture. The best stands of Timothy are obtained when seeds are sown not deeper than 1/2 inch in a well-prepared, firm seedbed. A firm seedbed is essential to the successful establishment of small-seeded grasses such as timothy. A firm seedbed allows greater regulation in seeding depth, holds moisture better, and increases seed-to-soil contact (Hall et al., 1914).

Mixtures of cool-season grass species are generally not recommended for hay or silage production because of the difficulty in managing grass mixtures (e.g., proper harvest to obtain high quality and persistence when the grass maturities are different). However, timothy is frequently planted in mixture with other grasses for use in pastures, especially pastures for horses (Hall et al., 1914).

Frequently cutting or grazing can easily reduce the growth of timothy. Timothy requires a high level of fertility for maximum production. Potassium fertilizer is important for maximizing the legume yield in a timothy-legume mixed stand (Hall et al., 1914). Table 1. show the apparent digestion co-efficient of timothy and the proximate composition of timothy respectively.

Timothy hay has been preferred as a feed for horses for many years. It is low in protein, thus. It is best to cut timothy at the pre-bloom stage in order to increase its protein content, decrease its fibre content, and increase its palatability and digestibility (Cunha, 1980).

**Table 1.3:** Apparent Dry Matter, Crude Protein and Crude Fibre percentages of Timothy (*Phleum pratense*)

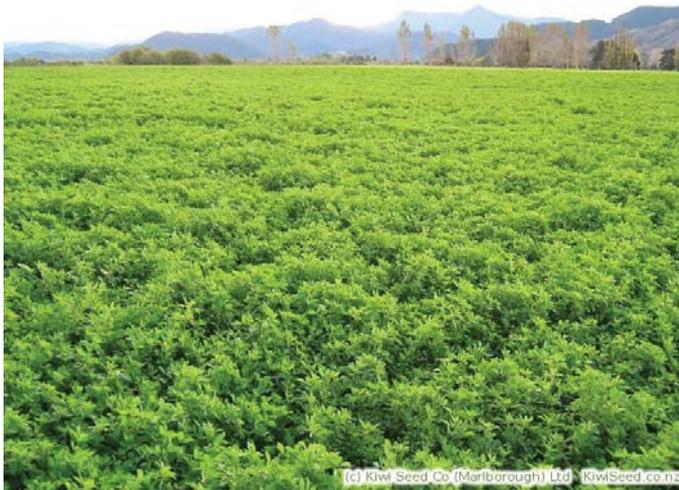
Stage of maturity	Dry Matter	Crude Protein	Crude Fibre
Pre Bloom	65.9	65.2	65.4
Early Bloom	60.6	62.1	59.7
Mid Bloom	59.3	55.3	57.9

After (Darlington et al., 1968)

#### 1.4 Lucerne as a feed for horses

Lucerne (*Medicago sativa, Lin.*), also known as alfalfa, is a member of the clover or leguminous plant family. Lucerne is a deep rooted legume that does best on deep, sandy, or light loam soil well-drained soil with a pH of 6.5 or higher (Lewis, 1995). Legumes such as lucerne have a symbiotic relationship with bacteria that enables them to utilise atmospheric and soil nitrogen for production of plant protein. The bacteria are usually contained in root nodules. Thus, legumes, particularly their leaves, are

typically much higher in protein, and also in calcium, than grasses (Lewis, 1995). Lucerne is a preferred forage for horses because of its high quality, high digestibility, and good roughage value (Shewmaker et al., 2005).



**Figure 1.3 :** Lucerne in the field (<http://kiwiseed.co.nz/lucerne.php> )

Lucerne is commonly grown on drought-prone soils in areas with 300-800 mm rainfall. North and central Otago steep land soils are particularly suited for lucerne (Langer, 1990)

Quality lucerne hay is high in protein, energy, vitamins, and minerals. Lucerne is highly digestible and usually contains more cell soluble material, less cellulose and hemicellulose, higher protein, lower fibre and has a higher relative feed value (RFV) than grass (Shewmaker et al., 2005). Feral horses adapted to wild lucerne which facilitated this plant's domestication as a specialized horse feed. Under conditions of

domestication, lucerne appears to offer horses several invaluable dietary advantages such as high protein content, and is much more suited to the horse than to man's other grazing herbivores (Small, 1996).

There are various factors affecting quality of lucerne, the most important being the stage of maturity at harvest. As the lucerne plant matures from the vegetative stage to flowering, the amount of fibre increases, while crude protein and digestibility decreases. Early cut lucerne hay is also more palatable and is consumed in larger quantities (Wilkinson, 2005).

### **1.5 Apparent Nutrient Digestibility**

Apparent nutrient digestibility is an important measurement in nutritional studies, it tells us the amount of nutrients the animal is digesting from its diet, and is calculated from the difference in the amount of nutrients in the diet and the amount remaining in the faeces or digesta. Digestibility studies using animals can determine the apparent or true digestibility of a feed or feed ingredient. The difference between the two terms is that apparent digestibility does not take into account endogenous nutrient and nitrogen losses, whereas true digestibility takes into account these losses. Apparent digestibility is measured through the total collection method, whereas true digestibility is measured through the ileal collection method. The two methods are similar; and involve feeding an animal a diet and either collecting all faeces produced over a given period, or collecting digesta from the ileum at a specific time over a given period respectively. The ileal collection method can be carried out using euthanasia or ileal cannulation protocols. This has been done in a number of species such as pigs, rabbits and dogs (Chung et al., 1992; Gidenne, 1992; Mroz et al., 1996; Muir et al., 1996).

The total faecal collection method, although only allowing apparent digestibility values to be determined, is straight forward and the most common method of establishing the digestibility of a feed or feed ingredient. The total collection protocol starts with an adjustment period during which the animal adapts to the diet. A measured amount of the diet is fed and all faeces are collected over the 'collection period'. There are a

number of protocols with different lengths of adaptation and collection periods. In digestion trials with equines, the total number of collection days for determining the digestibility of feedstuffs has been variable, the length of time being reported as 5 days by Pagan et al. (1998) and Paterson (1879), 7 days by Nicholson et al. (1965) and Sutton et al. (1977), 8 days by Lindsey et al. (1926), 10 days by Haenlein et al. (1966) and 12 days by Lathrop et al. (1938). Rate of passage of feedstuffs through the digestive tract of horses has been studied by Edin et al. (1929) and Olsson et al. (1949) and of ponies by Haenlein et al. (1966), but no definite conclusions were drawn regarding the specific number of collection days recommended for digestion trials.

The main problem with the total collection method is that it is time consuming and labour intensive. The animals need to be kept in individual pens and in an environment that ensures total recovery of faeces. However, when compared with other available methods, it is the least invasive and generally the cheapest method to use.

### **1.6 Voluntary Feed Intake**

Voluntary feed intake (VFI) exerts a greater influence on feeding value than nutritional value *per se* (Waghorn et al., 2004). Horses appear better than ruminants at adapting to fibrous forage of low nutritional value by increasing VFI and hence increasing energy intake (Janis, 1976). Indeed, although cattle are more efficient at dietary fibre degradation per unit fibre eaten, horses are more efficient per unit time due to increased dry matter intake (DMI) and passage rate (Hoskin et al., 2004). This indicates that herbage availability may need to be higher for maximum production from horses compared with cattle when forages are fibrous and of low nutritional value.

A limited number of studies have attempted to measure VFI of housed horses (Ordakowski-Burk et al., 2006; Ragnarsson et al., 2008; Ragnarsson et al., 2010a). Factors affecting VFI of horses have been recently reviewed, revealing a paucity of information available for fresh forages (Cuddeford et al., 2002). Horses and ponies rely more strongly on oropharyngeal and external stimuli for control of feed intake than other species (Ralston et al., 1983). In captivity at low densities horses perform latrine

behaviour, establishing a pattern of closely-grazed 'lawns' and un-grazed 'roughs' where horses prefer to defecate and urinate and not graze (Archer, 1978; Ödberg et al., 1977).

In order to measure VFI, herbage availability should not limit DMI. Little information is available regarding the effect of herbage allowance on VFI of horses for different pasture species or mixes (Hoskin et al., 2004). Seasonal changes in pasture composition and plant maturity are difficult to separate, but regardless of the season or species, horses tend to select the youngest, fastest-growing herbage (Hoskin et al., 2004), and possibly select for as high sugar, rather than low lignin content (Rogalski, 1984). Palatability studies with pure swards in different temperate regions of the world showed horses preferred Italian and hybrid ryegrasses, prairie grass (*Bromus cartharticus*), creeping red fescue (*Festuca rubra*) and tall fescue over perennial ryegrasses, timothy, brown top (*Agrostis tenuis*) and cocksfoot (Archer, 1978; McCann et al., 1991). However, when a pasture mix of tetraploid perennial ryegrass, other perennial grasses and white clover was included in addition to pure swards, the mix was preferred (Hoskin et al., 2004). Horses appeared to prefer white clover over other legumes (Hoskin et al., 2004).

Free ranging horses tend to show 10-15 distinct feeding bouts within 24 hours and spend around 10-14 hours per day displaying feed intake behaviour. Voluntary intake of dry matter ranges between 1.3-1.7% of BW for straws, 1.9% BW for hays and 2.6 % BW for fresh cut grass (Ellis et al., 2010).

Horses have evolved as grass eaters but, in temperate countries, pasture grass cannot be provided all year due to the cold climate. The grass therefore has to be conserved and traditionally hay-making has been the most common form of conservation. In recent years, hay has largely been replaced by silage and haylage in equine diets (Müller & Udén, 2007). Forages preserved by ensiling are usually harvested at an earlier stage of maturity than forages preserved as hay. This results in an increased digestibility nutrients and the energy value is markedly increased (Ragnarsson et al., 2008). As a consequence, less dry matter (DM) has to be fed to meet the energy

requirements of horses (Ragnarsson et al., 2010b). Although many opinions about the suitability of different conservation methods of grass to be used for horses exist, the scientific knowledge in the area is scarce, and reported work has mainly dealt with voluntary intake and digestibility effects on behaviour or effect of forage species on voluntary intake and digestibility (LaCasha et al., 1999; Müller & Udén, 2007). The nutritive value and digestibility of forages are important parameters, but only if the forage is not rejected by horses. Ensiling has been reported to reduce the voluntary intake of forages in other animal species (Buchanan-Smith, 1990; Erdman, 1993; Wilkins, 1974) and in horses (Moore-Colyer et al., 2000), and horse owners sometimes claim that silage will not be eaten by their horses.

## **1.7 Feeding and various different behaviours in Horses**

Horses have evolved as grazers. Their food sources; grasses, legumes and shrubs were continuously and relatively abundant but seasonally variable in both quality and quantity (Ralston, 1984). Free ranging horses and ponies graze 10 to 14 h/d in bouts of 2 to 3 h. Grazing bouts are separated by periods of resting and of locomotion or social activity (Francis-Smith, 1977; Tyler, 1972). The duration and frequency of grazing bouts are influenced by the quality of the available forages and climatic factor (Ralston, 1984; Tyler, 1972). During the grazing bouts the animals maintain fairly constant forward movement taking a few bites and then chewing while walking forward before ingesting more grass. They will linger longer on patches of preferred grasses and move more rapidly through areas of less palatable forages (Archer, 1971). The temporal pattern of grazing bouts is influenced by the quality of the available forages and climatic factors (Ralston et al., 1983).

It is not unusual for an entire herd to graze at the same time and in the same direction, maintaining an individual distance of at least one meter between member of the group. Social factors influence feeding in the stables. Sweeting et al (1985) observed that ponies in individual stalls spent considerable time feeding when they could maintain visual contact, the ponies reduced their feeding time (especially in the afternoon) and spent more time standing alert when they had no visual contact.

There is a distinct diurnal variation in feeding activity: larger, more frequent meals are consumed during the day (0800 to 1700 h) than at night (1700 to 0800 h) (Kern et al., 1972; Ralston et al., 1979). Thoroughbred horse are known to devote 53% of their available time to feeding in paddocks (Ralston, 1984). Neither horses nor ponies fast voluntarily for more than 3 to 5 h, the most prolonged fasts occurring between 0100 and 0600 h (Ralston, 1984).

### **1.7.1 Ingestive Behaviour**

Ingestive behaviour includes eating, drinking, food preference, daily patterns of feeding, the mechanics of obtaining food, and chewing food. Horses on pasture eat small amounts of feed all day long; horses kept in a stall eat at the convenience of the

owner or handler. If feed is freshly available horses have a tendency to overeat (Parker, 2008). Feeding behaviour is influenced by learned patterns and preferences, palatability of the feed, the environment, and social association.

Behaviour is a response to an organism's environment; the more restrictive an environment the more limited are the choices available to the organism. Choice is certainly reduced in feeding behaviour when horses are stabled. Feral or pastured horses may spend 70% of their day foraging, stabled horses on 'complete diets' may spend only 10% of their time feeding. These diets for competition or maintenance can be eaten in less than 2 h and have removed the feeding behaviour of stabled horses even further from its evolutionary origins (McGreevy, 2004).

The time at which food is made available often adheres to a strict regime, which may have variable implications for gastrointestinal function. The designation of such feeding times may address the convenience of the operator rather than the needs of the horse.

It has been argued that the sleep pattern of a species can be used as an index of adaptation to environment (Ruckerbusch, 1975). Standing-sleep behaviour is a state of drowsiness and indicative of slow-wave sleep, where the heart rate is decreased compared with heart rate during wakefulness (Waring, 2003). Standing-sleep may be induced to some extent when horses are less motivated to eat after consuming food. Therefore, Ninomiya et al. (2007) suggested that standing-sleep behaviour is a behavioural indicator of satisfaction and that it may be a useful indicator of horse welfare. It is therefore interesting that, while standing-sleep behaviour accounts for 8% of the resting time of stabled horses, this figure has been shown to rise to 14% at pasture (Ninomiya et al., 2007). The concomitant dietary differences between intake at grass and in the stable may play a role in this phenomenon since a preliminary study in ponies has shown that when oats replaced hay in the diet, total rest time increased (McGreevy, 2004). However, feeding and resting occupy most of the horse's day. As one increases the other decreases proportionally (Parker, 2008).

Ingestive behaviour includes drinking, which is relatively infrequent. Thirst is controlled by centres in the brain that work at the maintaining a specific level of body fluid. The control factors regulating thirst are influenced by hormones, salt intake, moisture content of the feed, and environmental factors (Parker, 2008).

Horses are adaptable to a variety of foods and indigestion schedules. They can tolerate environmental conditions with a scarcity of food and water; yet horses show a preference for grasses and grass-like plant material as well as for a nearby water source (McGreevy, 2004). Some stabled horses learn to moisten dry forage by “hay dunking”, placing their roughage into their water supply before chewing (McDonnell et al., 1999).

The times of day as well as the total time spent feeding are dependent on the quality and quantity of food available to horses, plus such factors as exercise, lactation, weather and insect pests (Waring, 2003). Environmental disruptions, such as storms or people, can temporarily cause horses to discontinue feeding. Social factors also influence feeding patterns; for example, as one horse begins to graze other group members are more inclined to graze.

Stabled horses fed limited amounts of concentrates; grains and hay generally consume their ration in one feeding bout and, thus, are unable to exhibit ingestive behaviour for the rest of the day. With food made constantly available, ponies were found to consume 80 per cent of their daily intake in an average of 10 separate meals (Ralston et al., 1979). It was also observed that quantity of feed and number of feedings can affect the behaviour of stabled horses as vacuum periods are formed during which the horses become restless and can easily be disturbed by an disturbance or provocation (McGreevy, 2004).

When given a choice, horses sometimes show preference for certain forms of foodstuff. Taste, texture, and odour clearly influence horse feeding; yet, feed intake as well as meal frequency are also influenced by gastrointestinal, metabolic, and environmental cues (Ralston, 1984). There appears to be no correlation between

energy content of a given feed and a horse's preference for that feed; nevertheless, based on hunger and external stimuli, the horse will regulate its body weight if given the opportunity to adapt to a single feed over a period of a few days (Ralston, 1986). It is not yet clear as to how genetics and environment affect feeding behaviour (Parker, 2008).

### **1.7.2 Abnormal Feeding Behaviour**

Aside from what can be considered a normal feeding behaviour, some horses spend time displaying abnormal feeding behaviours: ingesting faecal material (coprophagy), eating mud, or chewing wood (Waring, 2003). Coprophagy can be common in foals up to a month of age, but the foal weans thereafter. Usually one or two pellets are eaten subsequent to a bout of pawing the material. Coprophagy is rare in adult horses, although stallions are especially eager to investigate faeces and add to existing piles. Soil ingestion, although apparently not frequent, has been observed in horses under varying circumstances. Supplementation of dietary sodium has been suggested as the primary benefit of soil ingestion (Salter et al., 1980). Wood chewing, especially fence or stall chewing can be observed in some confined horses. Softwoods as well as hardwoods are vulnerable. Horizontal boards as well as upright boards at corners are the usual targets for chewing, whenever the animal stands for considerable time. Although some ingestion may occur, much of the chewed material falls to the ground (Waring, 2003).

### **1.7.3 Stereotypic Behaviour**

Stereotypic behaviour is characterised as repetitive, relatively invariant and apparently functionless behaviour. Stereotypies are heterogeneous in their cause and their forms. Historically known in horse-lore as 'stable vices' and given specific descriptive labels such as box walking, weaving, wind-sucking and crib-biting. A number of stereotypies have been identified, including: chewing, lip-licking, licking environment, wood-chewing, pawing, tail-swishing, door-kicking, box-kicking, rubbing self, self-biting, head-tossing, head-circling, head-shaking, head-nodding, head-extended, ears back and nodding, kicking stall (hind feet) (McGreevy, 2004).

Stereotypies are popularly regarded as being transmissible by mimicry and some are associated with health and performance problems. Horses exhibiting stereotypies are often isolated and it is theorised. There is a theory that stereotypies enable animals to cope with stress. The prevalence of crib-biting and weaving is greater in thoroughbreds than in other breeds (Luescher et al., 1998). It is difficult to dissect the effects of management and breed because Thoroughbreds are generally raced and therefore managed intensively (McGreevy, 2004).

#### **1.7.4 Agonistic Behaviour**

Agonistic behaviour includes fighting, flight, and other reactions associated with conflict (Parker, 2008). As a response to an environmental stimulus, a horse becomes alert and attempts to orientate the sensory receptors of the head towards the stimulus source. Recurring or minor sounds may cause only an ear to rotate while the horse continues to rest or forage. The alert posture, consisting of an elevated neck with intently oriented head and ears with nostrils sometimes slightly dilated, may induce similar alertness in neighbouring horses by looking, listening and smelling they test the situation. While being alert they open their eye-lids widely exposing white scleral tissue, fully elevate their neck, and dilate their nostrils and direct motion towards the stimulus. Defecation and nervous pawing may occur (McGreevy, 2004).

#### **1.7.5 Communicative Behaviour**

The horses are known to emit signals throughout the day that convey information. The information may pertain to the horse's intentions, present activity, social status, mood and emotions, identity, physiological conditions, or perhaps its awareness or concern about something in the surroundings. The horse itself may not be aware it is emitting such signals; yet, another animal may perceive and interpret any of the messages. The receiver may then emit signal relevant to the information it has just obtained and thus establish two-way communication (Waring, 2003).

Communicative signals between horses can be visual, acoustic, tactile or chemical. Interactions often involve more than one mode. Gradations of many expressive patterns occur depending upon the degree of stimulation and the situation. Communicative behaviour consists of:

- 1) Visual expression
    - Leg and body gestures
    - Facial expressions
    - Tail and other gestures
  - 2) Acoustical expressions
    - Squeal
    - Nicker
    - Neigh
    - Groan
    - Blow
    - Snort
    - Snore
    - Other sounds
  - 3) Tactile interactions
  - 4) Chemical exchanges
- (Waring, 2003)

### **1.8 How diets and various factors can affect the behaviour**

Studies have indicated that breed type, feeding regime, housing, and management conditions have a strong effect on developing abnormal stereotypic behaviour (Bachmann et al., 2003; Christie et al., 2006; McGreevy et al., 2010; Waters et al., 2002).

Horse owners have empirical evidence that diets high in energy tend to make horses excitable and inattentive to the handler (McCall, 2009). However, research investigating effects of different levels of dietary carbohydrates on spontaneous activity in a stall and reactivity to various startling stimuli could not conclusively show that high carbohydrate levels caused any increase in horse activity or excitability (Ralston et al., 1992). Holland et al. (1996) reported that adding 10 % fat to a typical hay/grain diet decreased reactivity of horses to various startling stimuli and reduced responses to pressure as compared to horses without added fat in the diet. This indicates that dietary fat may have a calming effect on horses and/or suggests that

excitability in the horse may be more related to the source of energy in the diet than the amount of energy (McCall, 2009). However, these studies, as well as the average horse owner, have trouble accurately measuring reactivity and ruling out extraneous factors such as inconsistent exercise and changing environmental conditions that might affect reactivity (McCall, 2009).

Providing *ad libitum* forage may give the horse a competing activity and provide gut fill, making it less anxious around feeding time and reducing the expression of locomotor stereotypies. Cribbing frequency increases around mealtimes (Gillham et al., 1994; Kusunose, 1992), and crib-biting behaviour is increased when adult horses are fed a low-forage and/or high-concentrate diet (McGreevy et al., 2010), sweetened grain rations (Gillham, et al., 1994), or if they are fed on an irregular basis (McClure et al., 1992). Horses on low-forage diets may fail to produce enough saliva to buffer stomach contents. Nicol (1999) proposed that cribbing may be an attempt to increase saliva flow to relieve gastric irritation.

A number of commercial supplements are marketed as calming agents for horses. These substances generally contain amino acids (especially tryptophan), B-vitamins, various minerals, and herbal ingredients. Although many horse owners routinely administer these products to horses before stressful situations, there is little scientific research validating the usefulness of these products (McCall, 2009). Bagshaw et al. (1994) reported that small doses of tryptophan (0.05 and 0.1 mg/kg) actually increased heart rates and activity in both non-stressed and stressed horses 2-4 hours after administration. Additionally they reported no differences in blood concentrations of serotonin, dopamine, or tryptophan among the treatments.

Lack of fibre in the diet has been implicated in behavioural problems such as wood chewing and tail chewing in the horse. Willard et al. (1977) reported that horses fed a total concentrate diet had a significantly lower caecal pH and spent significantly more time chewing wood than when on a hay diet. However, horses maintained on pasture still exhibit these behaviours, and it has been reported that there is no difference in

frequency of wood chewing behaviour in stalled horses given limited versus *ad libitum* hay (Gill et al., 1998).

Although wood chewing can be destructive to facilities, it may be a normal behaviour in horses. It seems to increase in cold, wet weather (Jackson et al., 1984) and may have evolved as a mechanism for horses to maintain body heat through activity or to maintain saliva flow without as much exposure to the elements as foraging for grasses.

Oral stereotypies (crib-biting/wind-sucking and wood-chewing) are mostly associated with the diet and the restriction of normal grazing behaviour (Bachmann et al., 2003; McGreevy et al., 2010; Waters et al., 2002), whereas locomotor stereotypies such as box-walking and weaving might be activated by social isolation or inadequate physical exercise (Bachmann et al., 2003; McGreevy et al., 2010). In horses, copying or observational learning as a mechanism of developing stereotypies is unlikely (Lindberg et al., 1999); however, social facilitation or stimulus enhancement cannot be ruled out.

A stereotypic neighbour spends less time eating and resting than a normal horse (McGreevy et al., 2010). As stereotypies are more frequent in disturbed environments, or when general activity of the horses is great (Cooper et al., 2000), constant movement during weaving, or sound production during wind-sucking by stereotypic horses can make sensitive individuals restless and induce abnormal stereotypic behaviour. There is only anecdotal evidence for a possible role of stimulus enhancement.

## **1.9 Behavioural Measurement**

### **1.9.1 Ethograms**

Equid behaviour has historically been a topic of great interest to animal keepers, biologists, animal scientists, veterinarians, and ethologists. Numerous descriptive accounts of equid behaviour as well as a few systematic studies focusing on particular species or types of behaviour are available in the lay and scientific literature. In this body of published information, there are notable apparent inconsistencies in the terminology and interpretations of behaviour.

An ethogram is a formal description of a species behavioural repertoire or a major segment of it. It may be a complete list of all behaviours or it may focus on particular functional classes of behaviours (Grier et al., 1984). Ethograms have been developed and used in a number of equine studies; to describe differences in play among domestic foals of different ages and gender among a population of domestic horses and ponies kept under semi-feral conditions in the New Forest area of England (Tyler, 1972), in Appaloosa horses pastured under semi-natural conditions in Idaho, USA (Blakeslee, 1974); in feral African wild asses of Death Valley, CA, USA (Moehlman, 1974), in a feral horse population off the coast of North Carolina, USA (Hoffmann, 1985); and in Przewalski horses to study time budgets in a semi-preserve in Virginia, USA (Boyd et al., 1988).

### **1.9.2 Scan Sampling**

Instantaneous sampling carried out on groups is known as scan sampling. It is a technique in which the observer records an individual's current activity at preselected moments in time (e.g. every minute in an hour session). Scan sampling can be used to obtain data from a large number of group members, by observing each in turn. Moreover, if the behaviours of all visible group (or subgroup) members are sampled within a very short time period the record approaches a simultaneous sample on all individuals (Altmann, 1974). If scan sampling is done frequently, data is obtained on the time distribution of behavioural states of the whole social group. A primary use of scan sampling is in studies of the amount or percent of time that individuals devote to various activities. The percent of time is estimated from the percent of samples in which a given activity (state) was recorded. Instantaneous scan samples are discrete samples of states, i.e., of ongoing behaviours. They are not samples of events, or transition times between states (Altmann, 1974).

There are various examples of scan sampling for behaviour in horses such as Cooper et al., 2005), where each stabled horse was scanned once every 2 min (30 scans per hour of observation) to observe short-term effects of increasing meal frequency on stereotypic behaviour. Other uses of the technique in horses include; measuring foal suckling behaviour (Cameron et al., 1999), transportation (Waran et al., 1996), the use

of mirrors to control stereotypic behaviour in stabled horses (McAfee et al., 2002), and the assessment of emotionality in horses (Wolff et al., 1997). This shows scan sampling can be used for various behaviours exhibited by horses.

### **1.10 Aim of the study**

Various studies have been carried out on the digestibility, voluntary feed intake and behaviour of thoroughbred horses on different feed types. However, no studies in New Zealand have investigated the difference in digestibility in haylage products containing timothy grass.

The aim of the current studies was to determine the digestibility and voluntary feed intake of two conserved forages in stabled horses. The specific objectives of the project were; (i) to determine the digestibility of FiberEzy® (a haylage made with the mixture of lucerne and timothy) and Timothy alone, when fed to thoroughbred horses; (ii) establish the Voluntary Feed Intake (VFI) of FiberEzy® versus Rye Clover hay; and (iii) undertake behavioural observations of thoroughbred horses during the voluntary feed intake period when they are fed FiberEzy® and Rye Clover hay under the same stabled conditions and feeding environment.

### **1.11 Hypotheses**

1. There is a significant difference in the digestibility of FiberEzy® and timothy.
2. There is a significant difference in the voluntary feed intake of FiberEzy® and rye clover hay between groups of horses on each feed.
3. There is a significant difference in the behaviour between groups of horses on each feed.

## References

- AgResearch. Timothy-Grasslanz Unique plant opportunities Retrieved 24/10/2012, from <http://www.grasslanz.com/LinkClick.aspx?fileticket=b5OXDbWHCVs%3D&tabid=78>
- Altmann, J. (1974). Observational Study of Behavior: Sampling Methods. *Behaviour*, 49(3/4), 227-267. doi: 10.2307/4533591
- Archer, M. (1971). Preliminary studies on the palatability of grasses, legumes and herbs to horses. *Veterinary Record*, 89(9), 236-240.
- Archer, M. (1978). Studies on Producing and Maintaining Balanced Pastures for Studs. *Equine Veterinary Journal*, 10(1), 54-59. doi: 10.1111/j.2042-3306.1978.tb02216.x
- Bachmann, I., AudigÉ, L., & Stauffacher, M. (2003). Risk factors associated with behavioural disorders of crib-biting, weaving and box-walking in Swiss horses. *Equine Veterinary Journal*, 35(2), 158-163. doi: 10.2746/042516403776114216
- Bagshaw, C., Ralston, S., & Fisher, H. (1994). Behavioral and physiological effect of orally administered tryptophan on horses subjected to acute isolation stress. *Applied Animal Behaviour Science*, 40(1), 1-12.
- Battle, G. H., Jackson, S., & Baker, J. (1988). Acceptability and digestibility of preservative-treated hay by horses. *Nutrition reports international*, 37(1), 83-89.
- Bels, V. L. (2006). *Feeding in domestic vertebrates : from structure to behaviour / edited by V. Bels*: Cambridge, MA : CABI Pub., c2006.
- Berg, C. C., McElroy, A. R., & Kunelius, H. T. (1996a). Timothy. *Cool-season forage grasses(coolseasonforag)*, 643-664.
- Berg, C. C., McElroy, A. R., & Kunelius, H. T. (1996b). Timothy1. *Cool-season forage grasses, agronomymonogra(coolseasonforag)*, 643-664. doi: 10.2134/agronmonogr34.c20
- Bergero, D., & Peiretti, P. G. (2011). Intake and Apparent Digestibility of Permanent Meadow Hay and Haylage in Ponies. *Journal of Equine Veterinary Science*, 31(2), 67-71. doi: 10.1016/j.jevs.2010.12.006
- Bergero, D., Peiretti, P. G., & Cola, E. (2002). Intake and apparent digestibility of perennial ryegrass haylages fed to ponies either at maintenance or at work. *Livestock Production Science*, 77(2-3), 325-329. doi: 10.1016/s0301-6226(02)00031-3
- Blakeslee, J. K. (1974). *Mother-young relationships and related behavior among free-ranging Appaloosa horses*: Idaho State University.
- Boyd, L. E., Carbonaro, D. A., & Houpt, K. A. (1988). The 24-hour time budget of Przewalski horses. *Applied Animal Behaviour Science*, 21(1), 5-17.
- Buchanan-Smith, J. (1990). An investigation into palatability as a factor responsible for reduced intake of silage by sheep. *Anim. Prod*, 50, 253-260.
- Burk, A. O., & Williams, C. A. (2008). Feeding management practices and supplement use in top-level event horses. *Comparative Exercise Physiology*, 5(02), 85-93. doi: doi:10.1017/S1478061508062786
- Byers, J. A. (1998). *Animal play: evolutionary, comparative and ecological perspectives*: Cambridge University Press.

- Cameron, E. Z., Stafford, K. J., Linklater, W. L., & Veltman, C. J. (1999). Suckling behaviour does not measure milk intake in horses, *Equus caballus*. *Animal Behaviour*, 57(3), 673-678. doi: <http://dx.doi.org/10.1006/anbe.1998.0997>
- Christie, J. L., Hewson, C. J., Riley, C. B., McNiven, M. A., Dohoo, I. R., & Bate, L. A. (2006). Management factors affecting stereotypes and body condition score in nonracing horses in Prince Edward Island. *Canadian Veterinary Journal*, 47(2), 136-143.
- Chung, T. K., & Baker, D. H. (1992). Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. *Journal of Animal Science*, 70(12), 3781-3790.
- Cooper, J. J., McAll, N., Johnson, S., & Davidson, H. P. B. (2005). The short-term effects of increasing meal frequency on stereotypic behaviour of stabled horses. *Applied Animal Behaviour Science*, 90(3-4), 351-364. doi: 10.1016/j.applanim.2004.08.005
- Cooper, J. J., McDonald, L., & Mills, D. S. (2000). The effect of increasing visual horizons on stereotypic weaving: implications for the social housing of stabled horses. *Applied Animal Behaviour Science*, 69(1), 67-83. doi: 10.1016/S0168-1591(00)00115-5
- Crowell-Davis, S. L. (1986). Spatial relations between mares and foals of the Welsh pony (< i> Equus caballus</i>). *Animal Behaviour*, 34(4), 1007-1015.
- Cuddeford, D. (1996). *Equine Nutrition*. Ramsbury, Marlborough: The Crowood Press Ltd.
- Cuddeford, D., Garnsworthy, P., & Wiseman, J. (2002). Equine nutrition: some unique features, functions and frailties of the digestive system of the horse. *Recent advances in animal nutrition 2002*, 269-318.
- Cunha, T. J. (1980). *Horse feeding and nutrition*: Academic Press, Inc.(London) Ltd.
- Darlington, J. M., & Hershberger, T. V. (1968). Effect of Forage Maturity on Digestibility, Intake and Nutritive Value of Alfalfa, Timothy and Orchardgrass by Equine. *Journal of Animal Science*, 27(6), 1572-1576.
- Davidson, N., & Harris, P. (2003). Nutrition and Welfare. In N. Waran (Ed.), *The Welfare of Horses* (Vol. 1, pp. 45-76): Springer Netherlands.
- Davis, Z. (2009). *Introduction to Horse Nutrition*: Blackwell Publishing Ltd.
- Divers, T. J., Bartholomew, R. C., Messick, J. B., Whitlock, R. H., & Sweeney, R. W. (1986). CLOSTRIDIUM-BOTULINUM TYPE-B TOXICOSIS IN A HERD OF CATTLE AND A GROUP OF MULES. [Article]. *Journal of the American Veterinary Medical Association*, 188(4), 382-386.
- Dodds, K. L. (1994). *Clostridium botulinum*. In: *Handbook of Foodborne Diseases*. USA: Marcel Decker Inc.
- Dutton, M. F., Westlake, K., & Anderson, M. S. (1984). The interaction between additives, yeasts and patulin production in silage. *Mycopathologia*, 87(20-33).
- Dyce, K. M., Sack, W. O., & Wensing, C. J. G. (2009). *Textbook of veterinary anatomy*: Saunders.
- Ellis, A., Longland, A., Coenen, M., & Miraglia, N. (2010). Biological basis of behaviour in relation to nutrition and feed intake in horses. *EAAP publication*, 128, 53-74.
- Ellis, A. D., & Hill, J. (2005). *Nutritional physiology of the horse*: Nottingham University Press.

- Erdman, R. (1993). *Silage fermentation characteristics affecting feed intake*. Paper presented at the National Silage Production Conference.
- Evans, J. W. (2000). *Horses - A Guide to Selection, Care and Enjoyment* (3rd ed.): WH Freeman and Company, New York, NY.
- Fagen, R. (1981). *Animal play behavior*: Oxford University Press New York.
- Fagen, R., & George, T. K. (1977). Play behavior and exercise in young ponies (*Equus caballus* L.). *Behavioral Ecology and Sociobiology*, 2(3), 267-269.
- Frame, J., & Harkess, R. D. (1987). The Productivity of 4 forage legumes sown alone and with each of 5 companion grasses. [Article]. *Grass and Forage Science*, 42(3), 213-223. doi: 10.1111/j.1365-2494.1987.tb02109.x
- Frame, J., Harkess, R. D., & Hunt, I. V. (1985). Effect of seed rate of red clover and of companion timothy or tall fescue on herbage production. *Grass and Forage Science*, 40(4), 459-465. doi: 10.1111/j.1365-2494.1985.tb01777.x
- Francis-Smith, K. (1977). Behavior patterns of horses grazing in paddocks. *Appl Anim Ethol*, 3, 292-293.
- Frandsen, R. D., Wilke, W. L., & Fails, A. D. (2009). *Anatomy and physiology of farm animals*: Wiley-Blackwell.
- Frape, D. (2004). *Equine Nutrition & Feeding* (3rd ed.). Oxford: Blackwell Publishing Ltd.
- Gidenne, T. (1992). Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. *British Journal of Nutrition*, 67(01), 133-146.
- Gill, A. M., Parker, A., Leak, S., & Lawrence, L. M. (1998). Effects of meal feeding frequency and roughage on behaviors of stabled horses. *Advances in Equine Nutrition*, 89-92.
- Gillham, S. B., Dodman, N. H., Shuster, L., Kream, R., & Rand, W. (1994). The effect of diet on cribbing behavior and plasma  $\beta$ -endorphin in horses. *Applied Animal Behaviour Science*, 41(3), 147-153.
- Grier, J. W., & Burk, T. (1984). *Biology of animal behavior*: Times Mirror/Mosby College Pub.
- Gudmundsdottir, K. B., Svansson, B., Aalbaek, B., Gunnarsson, E., & Sigurdarson, S. (2004). *Listeria monocytogenes* in horses in Iceland. *Veterinary Record*, 155(456-459).
- Haenlein, G. F. W., Holdren, R. D., & Yoon, Y. M. (1966). Comparative Response of Horses and Sheep to Different Physical Forms of Alfalfa Hay. *Journal of Animal Science*, 25(3), 740-743.
- Hailu, K., Bettey, R. L., Ardans, A., Galey, F. D., Daft, B. M., Walker, R. L., . . . Byrd, J. W. (1991). Clostridium botulinum type C intoxication associated with consumption of processed alfalfa hay cube in horses. *J. Am. vet. med. Ass.*, 199, 742-746.
- Hall, M. H., & Cherney, J. H. (1914). Timothy- Agronomy Facts 24. *PennState College of Agriculture-Cooperative Extension*.
- Hintz, H., & Cymbaluk, N. (1994). Nutrition of the horse. *Annual review of nutrition*, 14(1), 243-267.
- Hoffmann, R. (1985). On the development of social behaviour in immature males of a feral horse population (*Equus przewalskii* f. *caballus*). *Zeitschrift f̃r S̃ugetierkunde*, 50, 302-314.
- Holland, J., Kronfeld, D., & Meacham, T. (1996). Behavior of horses is affected by soy lecithin and corn oil in the diet. *Journal of Animal Science*, 74(6), 1252-1255.

- Hoskin, S. O., & Gee, E. K. (2004). Feeding value of pastures for horses. *New Zealand Veterinary Journal*, 52(6), 332-341. doi: 10.1080/00480169.2004.36449
- Houpt, K., Houpt, T. R., Johnson, J. L., Erb, H. N., & Yeon, S. C. (2001). The Effect of Exercise Deprivation on the Behaviour and Physiology of Straight Stall Confined Pregnant Mares. *Animal Welfare*, 10(3), 257-267.
- Jackson, S. A., Rich, V. A., Ralston, S. L., & Anderson, E. W. (1984). Feeding behavior and feed efficiency in groups of horses as a function of feeding frequency and the use of alfalfa cubes. *J. Anim. Sci*, 59(Suppl 1), 152.
- Janis, C. (1976). The Evolutionary Strategy of the Equidae and the Origins of Rumen and Cecal Digestion. *Evolution*, 30(4), 757-774.
- Jonsson, A. (1989). The role of yeasts and Clostridia in silage deteriorations. *Rapport nr. 42. Sveriges Lantbruksuniversitet*, 11-22. .
- Jørgensen, M., Schjelderup, I., & Junntila, O. (1994). Dry matter production and botanical composition of monocultures and mixtures of meadow fescue (*Festuca pratensis* Huds.) and timothy (*Phleum pratense* L.) in field experiments at three locations in northern Norway 1984-89. *Norwegian Journal of Agricultural Sciences*, 8.
- Kern, D., & Bond, J. (1972). Eating patterns of ponies fed diets ad libitum. *Journal of Animal Science Abstract*, 35, 285.
- Kunelius, H. T., Dürr, G. H., McRae, K. B., & Fillmore, S. A. E. (2006). Performance of Timothy-based Grass/Legume Mixtures in Cold Winter Region. *Journal of Agronomy and Crop Science*, 192(3), 159-167. doi: 10.1111/j.1439-037X.2006.00195.x
- Kunelius, H. T., Durr, G. H., McRae, K. B., Fillmore, S. A. E., Belanger, G., & Papadopoulos, Y. A. (2003). Yield, herbage composition, and tillering of timothy cultivars under grazing. [Article]. *Canadian Journal of Plant Science*, 83(1), 57-63.
- Kusunose, R. (1992). Diurnal pattern of cribbing in stabled horses. *Japanese Journal of Equine Science*, 3(2), 3.
- LaCasha, P. A., Brady, H. A., Allen, V. G., Richardson, C. R., & Pond, K. R. (1999). Voluntary intake, digestibility, and subsequent selection of Matua bromegrass, coastal bermudagrass, and alfalfa hays by yearling horses. *Journal of Animal Science*, 77(10), 2766-2773.
- Langer, R. H. M. (1990). *Pastures: their ecology and management*: Oxford University Press Auckland^ eNew Zealand New Zealand.
- Lathrop, A., & Bohstedt, G. (1938). Oat mill feed.
- Lawrence, L., JASTER, E., WISCHOVER, L., MOORE, K., & HINTZ, H. (1987). Acceptability of alfalfa hay treated with an organic acid preservative for horses. *Canadian Journal of Animal Science*, 67(1), 217-220.
- Lewis, L. D. (1995). *Equine Clinical Nutrition : Feeding and Care*. PA, USA: Williams & Wilkins.
- Lindberg, A. C., Kelland, A., & Nicol, C. J. (1999). Effects of observational learning on acquisition of an operant response in horses. *Applied Animal Behaviour Science*, 61(3), 187-199. doi: 10.1016/s0168-1591(98)00184-1
- Lindsey, J., Beals, C., & Archibald, J. (1926). The digestibility and energy values of feeds for horses. *J. Agr. Res*, 32, 569.

- Luescher, U. A., McKeown, D. B., & Dean, H. (1998). A cross-sectional study on compulsive behaviour (stable vices) in horses. *Equine Veterinary Journal*, 30(S27), 14-18. doi: 10.1111/j.2042-3306.1998.tb05138.x
- McAfee, L. M., Mills, D. S., & Cooper, J. J. (2002). The use of mirrors for the control of stereotypic weaving behaviour in the stabled horse. *Applied Animal Behaviour Science*, 78(2-4), 159-173. doi: 10.1016/s0168-1591(02)00086-2
- Mccall, C. (2009). Equine Behavior: A Nutritional Link? *Advances in equine nutrition*, Auburn University, Alabama.
- McCann, J., & Hoveland, C. (1991). Equine grazing preferences among winter annual grasses and clovers adapted to the southeastern United States. *Journal of Equine Veterinary Science*, 11(5), 275-277.
- McClure, S. R., Chaffin, M. K., & Beaver, B. V. (1992). Nonpharmacologic management of stereotypic self-mutilative behavior in a stallion. *Journal of the American Veterinary Medical Association*, 200(12), 1975.
- McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., & Morgan, C. A. (2002). *Animal nutrition* (6th ed.). Harlow: UK Prentice Hall.
- McDonnell, S. M., Freeman, D. A., Cymbaluk, N. F., Schott, H. C. I., Hinchcliff, K., & Kyle, B. (1999). Behavior of stabled horses provided continuous or intermittent access to drinking water. *American Journal of veterinary research*, 60(11), 1451-1456.
- McDonnell, S. M., & Haviland, J. C. S. (1995). Agonistic ethogram of the equid bachelor band. *Applied Animal Behaviour Science*, 43(3), 147-188.
- McDonnell, S. M., & Poulin, A. (2002). Equid play ethogram. *Applied Animal Behaviour Science*, 78(2-4), 263-290. doi: [http://dx.doi.org/10.1016/S0168-1591\(02\)00112-0](http://dx.doi.org/10.1016/S0168-1591(02)00112-0)
- McGreevy, P. (2004). *Equine Behaviour*. Philadelphia, USA: Elsevier.
- McGreevy, P., Cripps, P. J., French, N. P., Green, L. E., & Nicol, C. J. (2010). Management factors associated with stereotypic and redirected behaviour in the Thoroughbred horse. *Equine Veterinary Journal*, 27(2), 86-91.
- Meyer, H., Ahlswede, L., & Reinhardt, H. J. (1975). Time of feed uptake, masticatory frequency, and mincing of feed in horses. *UNTERSUCHUNGEN UBER FRESSDAUER, KAUFREQUENZ UND FUTTERZERKLEINERUNG BEIM PFERD*, 82(2), 54-58.
- Moehlman, P. D. R. (1974). *Behavior and ecology of feral asses (Equus asinus)*: University of Wisconsin--Madison.
- Moore-Colyer, M. J. S., & Longland, A. C. (2000). Intakes and in vivo apparent digestibilities of four types of conserved grass forage by ponies. *Journal of Animal Science*, 71, 527-534. doi: 1357-7298/00/07890527
- Moore-Colyer, M. J. S., Longland, A. C., & Morrow, H. J. (2003). Mathematical modelling of digesta passage rate, mean retention time and in vivo apparent digestibility of two different lengths of hay and big-bale grass silage in ponies. *British Journal of Nutrition*, 90, 109-118. doi: 10.1079/BJN2003869
- Mroz, Z., Bakker, G. C., Jongbloed, A. W., Dekker, R. A., Jongbloed, R., & van Beers, A. (1996). Apparent digestibility of nutrients in diets with different energy density, as estimated by direct and marker methods for pigs with or without ileo-cecal cannulas. *Journal of Animal Science*, 74(2), 403-412.

- Muhonen, S., Lindberg, J., Bertilsson, J., & Jansson, A. (2009). Effects on fluid balance, digestion and exercise response in Standardbred horses fed silage, haylage and hay. *energy*, 5, 7.
- Muir, H. E., Murray, S. M., Fahey, G. C., Merchen, N. R., & Reinhart, G. A. (1996). Nutrient digestion by ileal cannulated dogs as affected by dietary fibers with various fermentation characteristics. *Journal of Animal Science*, 74(7), 1641-1648.
- Müller, C. E., & Udén, P. (2007). Preference of horses for grass conserved as hay, haylage or silage. *Animal Feed Science and Technology*, 132(1–2), 66-78. doi: 10.1016/j.anifeedsci.2006.02.013
- Nicholson, J., & Friend, D. (1965). The digestibility of potato pulp protein by some species of farm animals. *Canadian Journal of Animal Science*, 45(3), 141-145.
- Nicol, C. (1999). Understanding equine stereotypies. *Equine Veterinary Journal*, 31(S28), 20-25.
- Ninomiya, S., Kusunose, R., Sato, S., Terada, M., & Sugawara, K. (2004). Effects of feeding methods on eating frustration in stabled horses. *Animal Science Journal*, 75(5), 465-469. doi: 10.1111/j.1740-0929.2004.00214.x
- Ninomiya, S., Sato, S., Kusunose, R., Mitumasu, T., & Obara, Y. (2007). A note on a behavioural indicator of satisfaction in stabled horses. *Applied Animal Behaviour Science*, 106(1–3), 184-189. doi: 10.1016/j.applanim.2006.06.011
- Notermans, S., Kozaki, S., & Vanschothorst, M. (1979). TOXIN PRODUCTION BY CLOSTRIDIUM-BOTULINUM IN GRASS. [Article]. *Applied and Environmental Microbiology*, 38(5), 767-771.
- NRC. (2007) *Nutrient Requirement of Horses* (6th ed.). Washington, DC: The National Academies Press.
- Ödberg, F. O., & Francis-Smith, K. (1977). Studies on the formation of ungrazed eliminative areas in fields used by horses. *Applied Animal Ethology*, 3(1), 27-34. doi: 10.1016/0304-3762(77)90068-2
- Olsson, N., Ruudvere, A., Abstracts, N., & Reviews, V. (1955). *The Nutrition of the Horse*, By N. Olsson and A. Ruudvere.
- Ordakowski-Burk, A. L., Quinn, R. W., Shellem, T. A., & Vough, L. R. (2006). Voluntary intake and digestibility of reed canarygrass and timothy hay fed to horses. *Journal of Animal Science*, 84(11), 3104-3109. doi: 10.2527/jas.2005-607
- Pagan, J. D., Harris, P., Brewster-Barnes, T., Duren, S. E., & Jackson, S. G. (1998). Exercise Affects Digestibility and Rate of Passage of All-Forage and Mixed Diets in Thoroughbred Horses. *The Journal of Nutrition*, 128(12), 2704S-2707S.
- Parker, R. (2008). *Equine Science* (3rd ed.). New York: Delmar publication.
- Ragnarsson, S., & Lindberg, J. E. (2008). Nutritional value of timothy haylage in Icelandic horses. *113*(2), 202-208.
- Ragnarsson, S., & Lindberg, J. E. (2010a). Nutritional value of mixed grass haylage in Icelandic horses. *Livestock Science*, 131(1), 83-87.
- Ragnarsson, S., & Lindberg, J. E. (2010b). ORIGINAL ARTICLE: Impact of feeding level on digestibility of a haylage-only diet in Icelandic horses. *Journal of Animal Physiology and Animal Nutrition*, 94(5), 623-627. doi: 10.1111/j.1439-0396.2009.00947.x
- Ralston, S. L. (1984). Controls of Feeding in Horses. *Journal of Animal Science*, 59(5), 1354-1361.

- Ralston, S. L. (1986). Feeding behavior. *The Veterinary clinics of North America. Equine practice*, 2(3), 609-621.
- Ralston, S. L., & Baile, C. A. (1983). Factors in the control of feed intake of horses and ponies. *Neuroscience & Biobehavioral Reviews*, 7(4), 465-470. doi: 10.1016/0149-7634(83)90024-6
- Ralston, S. L., & McKenzie, S. (1992). Effect of carbohydrate intake on behavioral reactivity and activity levels in horses. *J. Anim. Sci*, 70(Suppl 1), 159.
- Ralston, S. L., Van den Broek, G., & Baile, C. A. (1979). Feed Intake Patterns and Associated Blood Glucose, Free Fatty Acid and Insulin Changes in Ponies. *Journal of Animal Science*, 49(3), 838-845.
- Ricketts, S. W., Greet, T. R. C., Glyn, P. J., Ginnett, C. D. R., McAllister, E. P., McCaig, J., . . . Murray, L. G. (1984). 13 CASES OF BOTULISM IN HORSES FED BIG BALE SILAGE. [Article]. *Equine Veterinary Journal*, 16(6), 515-518.
- Rogalski, M. (1984). Effect of carbohydrates and lignin on preferences for and intakes of pasture plants by mares. *Roczniki Akademii Rolniczej w Poznaniu*, 27, 183-193.
- Ruckerbusch, Y. (1975). The hypnogram as an index of adaptation of farm animals to changes in their environment. *Applied Animal Ethology*, 2(1), 3-18. doi: 10.1016/0304-3762(75)90061-9
- Salter, R. E., & Pluth, D. J. (1980). Determinants of mineral lick utilization by Feral Horses. *Northwest Science*, 54, 109-118.
- Schilder, M. B., Hooff, J. A., Geer-Plesman, C. J., & Wensing, J. B. (1984). A quantitative analysis of facial expression in the plains zebra. *Zeitschrift für Tierpsychologie*, 66(1), 11-32.
- Shewmaker, G. E., Undersander, D., Lawrence, L. M., & Lacefield, G. D. (2005). Alfalfa: The High Quality Hay for Horses. Retrieved 15/03/2012, 2012, from [http://www.alfalfa.org/pdf/Alfalfa%20for%20Horses%20\(low%20res\).pdf](http://www.alfalfa.org/pdf/Alfalfa%20for%20Horses%20(low%20res).pdf)
- Small, E. (1996). Adaptations to herbivory in alfalfa (*Medicago sativa*). *Canadian Journal of Botany*, 74(6), 807-822.
- Sutton, E. I., Bowland, J. P., & McCarthy, J. F. (1977). STUDIES WITH HORSES COMPARING 4N-HCl INSOLUBLE ASH AS AN INDEX MATERIAL WITH TOTAL FECAL COLLECTION IN THE DETERMINATION OF APPARENT DIGESTIBILITIES. *Canadian Journal of Animal Science*, 57(3), 543-549. doi: 10.4141/cjas77-070
- Sweeting, M. P., Houpt, C. E., & Houpt, K. A. (1985). Social Facilitation of Feeding and Time Budgets in Stabled Ponies. *Journal of Animal Science*, 60(2), 369-374.
- Tyler, S. J. (1972). The behavior and social organization of the New Forest ponies. *Animal Behaviour Monographs*, 5 (2), 85-344.
- Vandenput, S., Istasse, L., Nicks, B., & Lekeux, P. (1997). Airborne dust and aeroallergen concentrations in different sources of feed and bedding for horses. *Veterinary quarterly*, 19(4), 154-158.
- Waghorn, G., & Clark, D. (2004). Feeding value of pastures for ruminants. *New Zealand Veterinary Journal*, 52(6), 320-331.
- Waran, N. K., Robertson, V., Cuddeford, D., Kokoszko, A., & Marlin, D. J. (1996). Effects of transporting horses facing either forwards or backwards on their behaviour and heart rate. *Veterinary Record*, 139(1), 7-11. doi: 10.1136/vr.139.1.7
- Waring, G. H. (2003). *Horse Behaviour* (2nd ed.). Norwich, New York, USA: William Andrew Publishing.

- Waters, A. J., Nicol, C. J., & French, N. P. (2002). Factors influencing the development of stereotypic and redirected behaviours in young horses: Findings of a four year prospective epidemiological study. *Equine Veterinary Journal*, 34(6), 572-579.
- Wilkins, R. (1974). *The nutritive value of silages*. Paper presented at the Proceedings of the Eighth Nutrition Conference for Feed Manufacturers, Butterworths, Nottingham.
- Wilkinson, J. M. (2005). *Silage*. Lincoln, United Kingdom: Chalcombe.
- Willard, J. G., Willard, J., Wolfram, S., & Baker, J. (1977). Effect of diet on caecal pH and Feeding behaviour of horses. *Journal of Animal Science*, 45(1).
- Wolff, A., Hausberger, M., & Le Sclan, N. (1997). Experimental tests to assess emotionality in horses. *Behavioural Processes*, 40(3), 209-221. doi: [http://dx.doi.org/10.1016/S0376-6357\(97\)00784-5](http://dx.doi.org/10.1016/S0376-6357(97)00784-5)

## **CHAPTER 2 – Field sampling of Timothy, Lab analysis of FiberEzy® and ensiled Timothy and a shelf storage trial of FiberEzy®.**

### **2.1 Abstract**

**AIM:** To monitor how timothy grass grows under New Zealand conditions, to analyse the nutrient content of an ensiled timothy product and an ensiled product combining timothy with lucerne (FiberEzy®) and to assess the storage characteristics of FiberEzy®.

**METHODS:** Grass samples were collected from a representative section (6m x 16m) of field in Reporoa, (Waikato, New Zealand) planted with timothy grass (*Phleum pratense* L.) on the 10<sup>th</sup> of March 2010 at Longitude 176°34'E, Latitude 38°39'S. The samples were collected at regular intervals to investigate changes in the dry matter (DM), acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin content of the crop. Once harvested and ensiled, a bag of timothy and FiberEzy® (a 50:50 timothy:lucerne mix) were analysed for total DM, percentage crude protein, percentage crude fat, percentage crude fibre, percentage ash, gross energy percentage hot water soluble carbohydrates, percentage pectin, percentage NDF, percentage acid ADF, percentage lignin and vitamin E content. A shelf life stability study of FiberEzy® was carried out by sub-sampling a bag of the product every 3 months and analysing for total DM, percentage crude protein, percentage hot water soluble carbohydrates and vitamin E (mg/kg) over 12 months of storage. A total of four samples were tested.

**RESULTS:** There was an increase (17.7-35.9%) in the dry matter content and lignin content (3.0-6.3%) of timothy over the various harvest periods. Lab analysis of FiberEzy® versus timothy showed that FiberEzy® had higher levels of crude protein, pectin, lignin, ash and vitamin E ( $p < 0.05$ ), and lower levels of crude fibre and NDF ( $P < 0.05$ ) than the timothy grass. During the storage trial, analysis of FiberEzy® showed an increase in the content of vitamin E (37.6-124.9mg/kg) over time.

**CONCLUSION:** The addition of lucerne to timothy grass in the ensiled FiberEzy® product resulted in higher crude protein levels and lower levels of fibre as expected. Shelf storage of FiberEzy® resulted in a significant increase in the vitamin E content over time due to microbial action in the ensiled forages.

## 2.2 Introduction

Timothy (*Phleum pratense* L.) is a tufted perennial grass; light grey-green, with rounded tillers, flat leaves and swollen bulb-like tiller bases. The foliage is dull and hairless with a short ligule but no auricle where the leaf blade joins the sheath. Its flower-head is a spike-like 'pipe-cleaner', which has prominent awn points. The inflorescence is a spike-like panicle, from which the grass obtained its old name of catstail (Langer, 1990). Seeds are small, rounded and pale. Timothy is a bunch-type, shallow-rooted, cool season grass. Its shallow root system, however, makes it unsuited to drought prone soils (Hall et al., 1914), and it is sensitive to high temperatures.

In cold-winter regions, forage production depends on hardy species such as timothy which is grown mainly for silage and hay (Berg et al., 1996a). Because of the development of new grazing-type timothy cultivars, it is also included in pasture mixtures (Kunelius et al., 2003). Timothy is most productive during spring and early summer under cool, long-day conditions with abundant moisture (Kunelius et al., 2006). Regrowth after defoliation is variable and usually slower than that of most other grass species. Growing timothy in a mixture with other forage grasses and legumes tends to increase the productivity and reliability of forage crops that are under environmental stress (Berg et al., 1996b; Frame et al., 1987; Frame et al., 1985; Jørgensen et al., 1994).

Timothy is known to be a very highly palatable and nutritious grass (Langer, 1990). To thrive it needs medium to high fertility soils and adequate soil moisture. Because of these requirements and owing to its palatability and late-spring growth, timothy does not successfully compete with ryegrass (Langer, 1990). Timothy is grown primarily as hay for horses but is frequently included in pasture mixtures. It is less competitive in a legume mixture than most sod-forming grasses and is often grown in a legume mixture for hay (Hall et al., 1914). Timothy can be slow to establish and may fail when weed competition is severe during establishment. It establishes slowly, but once established it thrives on heavy, damp soils or under irrigation (Langer, 1990). Timothy hay has

been preferred as a feed for horses for many years. It is generally low in protein, although cutting at the pre-bloom stage maximises its protein content, minimises its fibre content, and increases its palatability and digestibility (Cunha, 1980). Timothy is not currently grown commercially in New Zealand.

Alfalfa or lucerne is botanically the same plant (*Medicago sativa*, Lin.), and one of the clover or leguminous family. Lucerne is a deep rooted legume that does best on well-drained soil with a pH of 6.5 or higher. Lucerne requires deep, sandy, or light loam soil (Lewis, 1995). Legumes such as lucerne have a symbiotic relationship with bacteria that enables them to utilise atmospheric and soil nitrogen for production of plant protein. The bacteria are usually contained in the root nodules. Thus, legumes, particularly their leaves, are much higher in protein, and also calcium, than grasses (Lewis, 1995). Lucerne is often the preferred forage for horses because of its high quality, high digestibility, and good roughage value (Shewmaker et al., 2005). Lucerne is commonly grown on drought-prone soils in areas with 300-800 mm rainfall. North and central Otago steep land soils are particularly suited for lucerne (Langer, 1990)

Quality lucerne hay is high in protein, energy, vitamins, and minerals. Lucerne is highly digestible and usually contains more cell soluble material, less cellulose and hemicellulose, higher protein, lower fibre and has a higher relative feed value (RFV) than grass (Shewmaker et al., 2005). Feral horses adapted to wild lucerne facilitated this plant's domestication as a specialized horse feed. Under conditions of domestication, lucerne appears to offer horses several dietary advantages such as high protein content, and is much more suited to the horse than to other domesticated grazing herbivores (Small, 1996).

In New Zealand, Lucerne is commonly used and fed as an ensiled product, and there appears to be a market demand for an ensiled lucerne/timothy product. However, under New Zealand conditions there is no data on growth of Timothy grass and its nutrient composition and the effect of storage on a commercially ensiled timothy and lucerne product.

## 2.3 Methods and materials

### 2.3.1 Sample collection of Timothy

A single field (591 m x 160 m) was sown with timothy grass (*Phleum pratense* L.) on the 10<sup>th</sup> of March 2011 by FiberFresh Feeds Ltd, Reporoa, Waikato (Longitude 176°34'E, Latitude 38°39'S). The timothy was grown to produce FiberEzy<sup>®</sup> (a 50:50 ensiled timothy/lucerne horse mix) as well as a 100% timothy haylage product.

Samples of the timothy grass (~1.5 kg wet weight) were collected from a central strip of the field (6m x 16m) which was measured out and marked while the crop was growing on 5<sup>th</sup> December, 2011. Fresh timothy grass samples were collected at a 15 day intervals (see figure 2.1) pre-harvest (T-15: 5<sup>th</sup> December, 2011), at harvest (T0: 20<sup>th</sup> December 2011), 15 days post-harvest (T15: 5<sup>th</sup> January 2011) and 45 days post-harvest (T45: 8<sup>th</sup> February 2012).

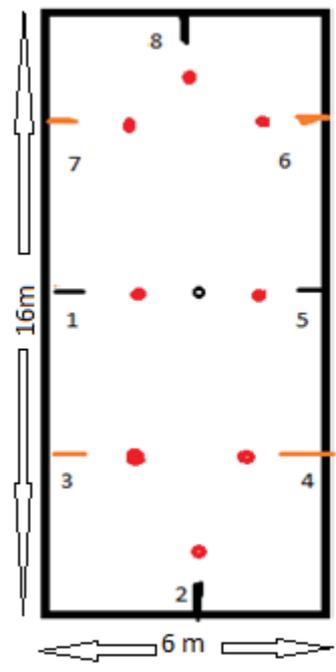
**Figure 2.1:** Timothy grass within the sample area (a) T-15: 15 days before harvest, (b) T0: at harvest, (c) T15: 15 days post-harvest and (d) T45: 45 days post-harvest



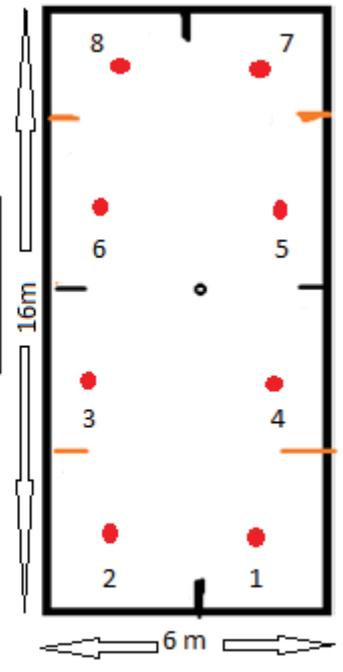
Eight samples were collected from positions in the central area at the four time points as shown in figure 2.2.

**Figure 2.2:** The areas where the sample was collected from (a) 15 days pre-harvest, (b) at harvest, (c) 15 days post- harvest and (d) 45 days post-harvest

(a)

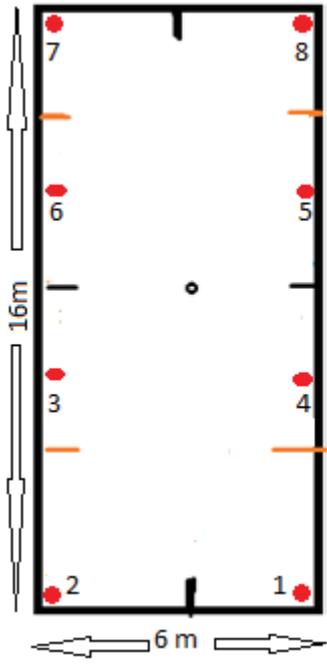


(b)

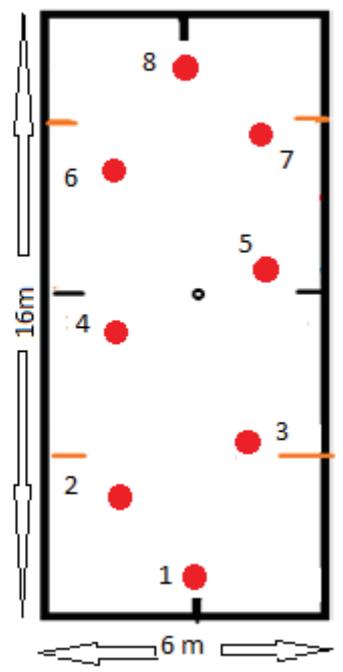


●	area where samples were collected from
—	area marking posts

(c)



(d)



●	area where samples were collected from
—	area marking posts

The fresh samples once collected were weighed, vacuum packed and transported for analysis at the Nutrition Laboratory (Institute of Food, Nutrition & Human Health, Massey University, Palmerston North) for dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and Lignin. Dry matter content was measured by drying samples in a convection oven at 105°C (WATVIC, Wellington, NZ). Total nitrogen (N) was determined by the Dumas method (Dumas, 1831) (Leco CNS 2000 Model 602 600 200) and was converted to CP by multiplying N by 6.25 (AOAC 968.06).

### **2.3.2 Analysis of ensiled FiberEzy® and Timothy**

Fresh bags of FiberEzy® and 100% timothy haylage were analysed for total DM, macronutrient content (percentage CP, percentage crude fat, percentage crude fibre, percentage ash, gross energy), micronutrient content (percentage hot water soluble carbohydrates, percentage pectin, percentage NDF, percentage ADF, percentage lignin, and vitamin E content).

### **2.3.3 Shelf storage of FiberEzy®**

A storage trial was also carried out to evaluate the loss of nutrients in FiberEzy® (50:50 mix of lucerne and timothy) stored at room temperature (22°C) over a twelve month period. Four bags of single batch of FiberEzy® (batch number- 070901011-21) were provided by FiberFresh Feeds Ltd. The bags were stored in the dark, and one was opened immediately (0 month: 4<sup>th</sup> August 2011), at 3 months (4<sup>th</sup> November 2011), 6 months (4<sup>th</sup> February 2011) and at 12 months (4<sup>th</sup> August 2012). At sampling each bag was opened and a representative sub-sample was collected from the bag for the analysis.

The FiberEzy® was analysed for total DM, percentage CP, percentage hot water soluble carbohydrates and vitamin E (mg/kg). Dry matter content was measured by drying samples in a convection oven at 105°C (WATVIC, Wellington, NZ). Hot water soluble

carbohydrates (HWSC) and pectin were extracted by the method of Blumenkrantz and Asboe Hansen (1973).

## 2.4 Results

### 2.4.1 Field sampling of Timothy:

Table 2.1 shows the results of the analysis carried out on the samples of timothy grass collected 15 days pre-harvest (5<sup>th</sup> December, 2011) to 45 days post-harvest (8<sup>th</sup> February 2012). There was a significant increase in DM content and lignin content of the grass during the collection period ( $p < 0.05$ ).

**Table 2.1:** Analysis of field samples of timothy at different periods of harvesting for dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin

Day of Sampling	Number of Samples	DM %	NDF %	ADF %	Lignin %
-15	8	17.7	62.1	34.9	3.0
0	8	21.4	62.0	36.7	3.9
+15	8	22.1	66.7	39.7	4.9
+45	8	35.8	62.0	35.4	6.3

### 2.4.2 Nutrient composition of FiberEzy® and Timothy

Table 2.2 shows the results of the analysis carried out on the bags of FiberEzy® and timothy grass opened on 5<sup>th</sup> April 2012. FiberEzy® had higher levels of crude protein, pectin, lignin, ash and vitamin E ( $p < 0.05$ ), and lower levels of crude fibre and neutral detergent fibre ( $P < 0.05$ ) than the timothy grass.

**Table 2.2:** Nutrient composition of FiberEzy® and Timothy (dry matter (DM) basis unless stated otherwise)

Item	FiberEzy®	Timothy
% Moisture( as is)	61.7	59.6
% Crude Protein	17.6	9.7
% Crude Fat	3.0	3.0
% Crude Fibre	31.5	39.6
% ADF	31.0	34.0
% NDF	49.7	63.2
% HWSC	4.9	5.3
% Pectin	3.3	0.8
% Lignin	6.0	5.1
% Ash	9.4	6.3
Gross Energy kJ/g	19.0	19.2
Vitamin E mg/kg	14.6	9.6

#### 2.4.3 Storage trial of FiberEzy®

Table 2.3 shows the results of the analysis carried out on the bags of FiberEzy® opened between 4<sup>th</sup> August 2011 and 4<sup>th</sup> August 2012. There was a significant increase in vitamin E content during the storage period ( $p < 0.05$ ).

**Table 2.2:** Selected nutrient composition of FiberEzy® during a 12 month storage trial (on a dry matter (DM) basis)

Date Sampled	Period	Number of Samples	Dry matter %	Crude protein %	HWSC %	Vitamin E mg/kg
4/08/2011	0 m	1	42.0	19.2	6.3	37.6
4/11/2011	3 m	1	41.7	18.8	6.5	46.2
4/02/2012	6 m	1	40.4	18.4	7.7	82.3
4/08/2012	12 m	1	41.3	18.9	5.9	124.9

During the storage trial, the bags of FiberEzy® were stored in the Nutrition Laboratory store room (Massey University, Palmerston North) and opened when required for analysis. On opening mould growth was observed on the surface of the product (see Figure 2.3), although the haylage underneath appeared to unaffected and still of good quality.

**Figure 2.3** Mould growth on bags of FiberEzy® during storage at room temperature in the Nutrition Laboratory (Massey University, Palmerston North) after (a) 3 months of storage, (b) 6 months of storage, (c) 12 months of storage.

(a) A FiberEzy® bag after 3 months storage at room temperature.



(b) FiberEzy® bag after 6 months storage at room temperature.



(c) FiberEzy® bag after 12 months storage at room temperature.



## **2.5 Discussion**

### **2.5.1 Field sampling of timothy.**

In the study, the timothy grass did not appear to grow to its full height as the soil and environment in Reporoa is different to the natural requirement of the grass. Its shallow root system makes it unsuited to drought prone soils (Hall et al., 1914), and it is sensitive to high temperatures. Timothy is most productive during spring and early summer under cool, long-day conditions with abundant moisture (Kunelius et al., 2006). Rainfall was not consistent during the growth period which could have affected the performance of the grass. In addition, timothy can be slow to establish and may fail when weed competition is severe during establishment. Given that this was the first time timothy was sown in the Reporoa area, better growth and performance may be observed in subsequent seasons when the crop is more established.

### **2.5.2 Storage Trial**

At present there are limited data on the effects of long-term storage on the breakdown of different components of ensiled forage over time. Water soluble carbohydrates and CP were selected for analysis to determine if they were destroyed by microbial activity and vitamin E was included due to its relative instability when exposed to light and rapid oxidation in air. Dry matter was analysed to provide a link to the results seen in the other nutrients.

There was limited variation in the percentage DM over the 12 month period. However there was some mild variation in the CP and HWSC percentage. Moreover, there was an increase in the content of vitamin E in the product over the 12 month storage period. The present study showed an increase in vitamin E from 0 to 12 months, which indicates that vitamin E levels increase with storage time. Fresh and conserved forages are a source of vitamins A, E, D, niacin and thiamine. The vitamins present in the forages are highly variable and the factors responsible for this variability could be due to plant origin, climatic conditions, stages of maturity, conservation methods (drying, ensiling, and dehydration) and storage conditions (Ballet et al., 2000). The level of  $\alpha$ -

tocopherol (a form of Vitamin E) in forage depends primarily on the stage of maturity, since mature plants have lower leaf: stem ratio than younger plant (Miller, 1958; Müller et al., 2007), and leaves contain higher levels of  $\alpha$ -tocopherol than stems (Brown, 1953; Horvath et al., 2006). Thus both plant species and stage of maturity has to be taken into consideration when comparing the influence of different conservation methods on  $\alpha$ -tocopherol content (Müller et al., 2007). On the other hand, it was found that losses of vitamin E occur in air-tight stored barley (Hakkarainen et al., 1983). This contradicts the present result where vitamin E content increases with storage time. An experiment conducted by (Müller et al., 2007) showed a positive correlation between  $\alpha$ -tocopherol content and lactic acid, acetic acid and clostridia, whereas a negative correlation was found between  $\alpha$ -tocopherol and DM, pH and water soluble carbohydrates. Vitamin E ( $\alpha$ -tocopherol) is more stable in acidic than in basic environment, and that it is relatively stable to heat and light in the absence of oxygen (Müller et al., 2007). This suggests that microbial action and the compounds produced by the microbes in the product may be contributing to the increased vitamin E levels observed in the product over time. Vitamin E is an important antioxidant and is vital for the humoral immune response in horses (Baalsrud et al., 1986). Vitamin E is destroyed by oxidation, which is enhanced by heat and UV light (Seshan et al., 1942). The length of drying or wilting period in the field affects the level of  $\alpha$ -tocopherol in hay and haylage or other wilted forage (Seshan, et al., 1942). The amount of vitamin E in international units (IU) can be derived by multiplying the content of  $\alpha$ -tocopherol in forage by 1.49 (NRC, 1989). According to NRC (1989), horses on maintenance level require 50IU/kg DM in total ration.

There is limited published data in the area of vitamin E production by microbes. However, vitamin E levels increased in the product with increasing mould growth on the product surface. This requires further investigation.

There is an increasing interest in non-structural carbohydrate concentrations in equine feeds because of the belief that high non-structural carbohydrate levels increase the risk of metabolic conditions in horses (Ralston, 1996; Treiber et al., 2005; Valentine et

al., 2001). There was no evidence that HWS carbohydrate levels increased with storage in the current work.

## 2.6 References:

- Baalsrud, K. J., & Overnes, G. (1986). Influence of vitamin E and selenium supplement on antibody production in horses. *Equine Veterinary Journal*, 18(6), 472-474.
- Ballet, N., Robert, J., & Williams, P. (2000). 19 Vitamins in Forages. *Forage Evaluation in Ruminant Nutrition*, 399.
- Berg, C. C., McElroy, A. R., & Kunelius, H. T. (1996a). Timothy. *Cool-season forage grasses(coolseasonforag)*, 643-664.
- Berg, C. C., McElroy, A. R., & Kunelius, H. T. (1996b). Timothy1. *Cool-season forage grasses, agronomymonogra(coolseasonforag)*, 643-664. doi: 10.2134/agronmonogr34.c20
- Brown, F. (1953). The tocopherol content of farm feeding-stuffs. *Journal of the Science of Food and Agriculture*, 4(4), 161-165.
- Cunha, T. J. (1980). *Horse feeding and nutrition*: Academic Press, Inc.(London) Ltd.
- Frame, J., & Harkess, R. D. (1987). The Productivity of 4 forage legumes sown alone and with each of 5 companion grasses. [Article]. *Grass and Forage Science*, 42(3), 213-223. doi: 10.1111/j.1365-2494.1987.tb02109.x
- Frame, J., Harkess, R. D., & Hunt, I. V. (1985). Effect of seed rate of red clover and of companion timothy or tall fescue on herbage production. *Grass and Forage Science*, 40(4), 459-465. doi: 10.1111/j.1365-2494.1985.tb01777.x
- Hakkarainen, R. J., Työppönen, J. T., & Bengtsson, S. G. (1983). Relative and quantitative changes in total vitamin E and isomer content of barley during conventional and airtight storage with special reference to annual variations. *Acta Agriculturae Scandinavica*, 33(4), 395-400.
- Hall, M. H., & Cherney, J. H. (1914). Timothy- Agronomy Facts 24. *PennState College of Agriculture-Cooperative Extension*.
- Horvath, G., Wessjohann, L., Bigirimana, J., Jansen, M., Guisez, Y., Caubergs, R., & Horemans, N. (2006). Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. *Phytochemistry*, 67(12), 1185-1195.
- Jørgensen, M., Schjelderup, I., & Junttila, O. (1994). Dry matter production and botanical composition of monocultures and mixtures of meadow fescue (*Festuca pratensis* Huds.) and timothy (*Phleum pratense* L.) in field experiments at three locations in northern Norway 1984-89. *Norwegian Journal of Agricultural Sciences*, 8.
- Kunelius, H. T., Dürr, G. H., McRae, K. B., & Fillmore, S. A. E. (2006). Performance of Timothy-based Grass/Legume Mixtures in Cold Winter Region. *Journal of Agronomy and Crop Science*, 192(3), 159-167. doi: 10.1111/j.1439-037X.2006.00195.x
- Kunelius, H. T., Durr, G. H., McRae, K. B., Fillmore, S. A. E., Belanger, G., & Papadopoulos, Y. A. (2003). Yield, herbage composition, and tillering of timothy cultivars under grazing. [Article]. *Canadian Journal of Plant Science*, 83(1), 57-63.
- Langer, R. H. M. (1990). *Pastures: their ecology and management*: Oxford University Press Auckland New Zealand.
- Lewis, L. D. (1995). *Equine Clinical Nutrition : Feeding and Care*. PA, USA: Williams & Wilkins.

- Miller, D. F. (1958). *Composition of cereal grains and forages* (Vol. 585): National Academies.
- Müller, C. E., Möller, J., Jensen, S. K., & Udén, P. (2007). Tocopherol and carotenoid levels in baled silage and haylage in relation to horse requirements. *Animal Feed Science and Technology*, 137(1–2), 182-197. doi: 10.1016/j.anifeedsci.2006.10.007
- NRC. (1989) *Nutrient Requirements of Horses* (6th ed.). Washington DC . The National Academy Press.
- Seshan, P., & Sen, K. (1942). Studies on Carotene in relation to animal nutrition: Part III. Stability of carotene in plant material with special reference to hay making and storage. *The Journal of Agricultural Science*, 32(03), 275-285.
- Shewmaker, G. E., Undersander, D., Lawrence, L. M., & Lacefield, G. D. (2005). Alfalfa: The High Quality Hay for Horses. Retrieved 15/03/2012, 2012, from [http://www.alfalfa.org/pdf/Alfalfa%20for%20Horses%20\(low%20res\).pdf](http://www.alfalfa.org/pdf/Alfalfa%20for%20Horses%20(low%20res).pdf)
- Small, E. (1996). Adaptations to herbivory in alfalfa (*Medicago sativa*). *Canadian Journal of Botany*, 74(6), 807-822.

## **CHAPTER THREE – Apparent digestibility of FiberEzy® and Timothy (*Phleum pratense*) haylage in Thoroughbred horses**

### **3.1 Abstract**

**AIMS:** To determine the apparent digestibility of dry matter (DM) and gross energy (GE) in ensiled FiberEzy® (50:50 lucerne and timothy) and timothy (*Phleum pratense*) haylage fed to four Thoroughbred horses.

**METHOD:** FiberEzy® and timothy haylage was offered at 2.5% of the body weight as DM to four Thoroughbred horses (10 ± 1 yr.; 562.5± 30.7 kg initial BW). The horses were stalled individually in loose boxes (4 x 4 m) lined with rubber matting. Horses were randomly paired and assigned to be fed FiberEzy® (FE) or timothy haylage (T) for two 18 day periods, in a 2 x 2 Latin square experimental design. Each 18 day period comprised of a 14 day dietary adaptation period where the horses were habituated to the pens and diet and a 4 d (96 h) total faecal collection. The apparent digestibility of DM and GE were measured on days 15-18; at 12 hourly intervals faecal material from each horse was collected, weighed, and a subsample was taken and frozen at -20°C. One kg samples of the feed were collected and frozen at -20°C on day 15.

**RESULTS:** The energy content of the FE and T varied between the first and second part of the study, with the DE of T varying from 6.9 MJ/kg DM to 9.4 MJ/kg DM. Total energy intake of FE and T varied between horses, and between groups, with horses fed T after FE reducing their total energy intake. One of these horses developed diarrhoea when switched to timothy, and was removed from the study.

**CONCLUSION:** The apparent digestibility of FiberEzy® tended to be greater than for timothy haylage.

### 3.2 Introduction

Timothy hay is a popular feed for horses, especially in North America, that is relatively low in protein and high in fibre content compared to other some other hays. Ideally timothy is cut at the pre-bloom stage in order to increase its protein content, decrease its fibre content, and increase its palatability and digestibility (Cunha, 1980). Lucerne (*Medicago sativa*), also known as alfalfa, is another popular forage for feeding horses (Crozier et al., 1997; NRC, 1989; Small, 1996). Lucerne is higher in protein, vitamins and some minerals than grasses (NRC, 1989) and is suitable to feed to horses of all ages and stages of production (Crozier et al., 1997; NRC, 1989).

Forages may be conserved for later use when there is little fresh forage available or when animals are confined to stalls. Such conservation is achieved by drying, ensiling, or applying preservative (NRC, 2007). Forages preserved by ensiling are usually harvested at an earlier stage of maturity than forages preserved as hay. This results in an increase in the digestibility of nutrients and the energy value (Ragnarsson et al., 2008). Conserved forages can be chopped, ground and pelleted, cubed, or wafered after drying, whereas ensiled forages are either bulk stored in bunkers or silos, or baled and wrapped in plastic film (NRC, 2007).

When the availability of pasture is limited, grass hay is traditionally the most common conserved forage that is offered to horses (Bergero, et al., 2011; Moore-Colyer et al., 2000). Hay is the most traditional method of conservation of grass. As long as hay is composed of safe, nontoxic, nutritious plants, the stage of maturity of the crop at the time of cutting, the weather conditions and care to which hay making is subject are much more important characteristics than the species of plant present (Frape, 2004). Timothy hay is generally composed of more stalk and less leaf compared to meadow hay and it also feels hard to touch. Hay tends to be less nutritious than haylage as it is cut later when the plant is in a more mature state. The quality and quantity of field dried hay for harvesting will depend on several factors such as stage of maturity when cut, methods of handling the hay, moisture content present in the hay and weather conditions during drying (Frape, 2004).

In recent years the inclusion of silage and haylage in equine diets has become more common (Müller & Udén, 2007). Haylage is a well conserved feed compared to hay and, the nutritive value is similar to that of original grass. Ensiled forages are moist, and therefore less dusty than hay.(Bergero et al., 2011; Moore-Colyer et al., 2000; Müller, 2005) and allergens that stabled horses in particular are exposed to (Vandenput, et al., 1997). Thus, ensiled forages may be recommended in the diets of horses with respiratory disease. Haylage can be packed in small bales for horses, so it can be consumed quickly after opening to prevent aerobic deterioration (Bergero et al., 2011). These small bales are popular with owners of low numbers of horses (Müller, 2005; Müller & Udén, 2007). It is important to ensure high quality preservation of silage and haylage as horses tend to use their sense of smell to select what to eat and will reject poorly conserved products (Wilkinson, 2005).

Ensiled forages are viewed with caution as a horse feed due to concerns of potential microbial contamination with *Clostridia* spp. or *Listeria* spp. The anaerobic condition of the ensiled forages favours the growth of the botulinum bacteria. The endospores are more tolerant to heat, irradiation, oxygen and antibiotics compared to other bacteria (Lewis, 1995). However, provided that ensiled forages are conserved and stored correctly, they do they not pose a risk to horse health and they are a valid alternative to hay. Anecdotal evidences suggest that ensiled grass forages are being fed to horses by an increasing number of horse owners with no detrimental effects (Moore-Colyer et al., 2000).

Haylage has a higher dry matter content than silage, leading to a higher pH. Clostridial multiplication is inhibited below pH 4.6. It has been shown that proteolytic strains of *Clostridium botulinum* type B can produce toxins in grass silage (Notermans et al., 1979). Noncarrion-associated botulinum (forage poisoning) of animals is reported and type B has frequently been demonstrated in such outbreaks (Divers et al., 1986). Wilting may inhibit growth of *Clostridium botulinum* as the vegetative bacteria is quite sensitive to high osmotic pressure, but safe values are difficult to estimate (Hailu et al., 1991; Jonsson, 1989). If the dry matter content of the silage is low (15% or below), the

pH may fall in the beginning of the process and increase the possibility of bacterial growth and toxin production (Dodds, 1994; Jonsson, 1989; Notermans et al., 1979).

The voluntary feed intake (VFI) of ensiled forages by horses is largely influenced by forage type and the DM content. Horses tend to have a larger VFI of high DM (40-65% DM) ensiled forages than low DM (<30% DM) ensiled forages (NRC, 2007). There are few data published on intake or apparent digestibility of forages in Thoroughbred horses and no data on feeding horses FiberEzy® and timothy haylage. The aim of this study was to determine the VFI, and DM and gross energy (GE) apparent digestibility of FiberEzy® and timothy haylage in Thoroughbred horses. The study also aimed to determine the duration of total faecal collection required to ensure consistent measurements of DM, CP and GE apparent digestibility.

### **3.3 Methods and materials**

#### **3.3.1 Animals**

Four Thoroughbred geldings ( $10 \pm 1$  yr.;  $562.5 \pm 30.7$  kg initial BW), housed at the LWT Equine Research Unit (Kiwitea, New Zealand), were used for the experiment. The horses were kept in rubber matted box stalls (4 m x 4 m). Immediately prior to start of the trial the horses had a basic clinical examination which included heart rate and respiration rate which were recorded and compared to the normal values. A faecal egg count was done on the four horses and on the basis of the results 2 horses were dewormed using Equest Plus (moxidectin and praziquantel; Pfizer Animal Health), administered orally. If required the hooves were trimmed by a farrier. Horses were weighed using a live-stock platform scale (Tru-test, EziWeigh2) at weekly intervals and a body condition score was assigned after Henneke et al. (1983). The horses were exercised individually or in pairs on a lime stone pressed yard outside the stalls (24 m by 8 m approx.), 30 min to an hour daily in the morning before feeding at 0800 h and in the evening before feeding at 2000 h. The horses were given no additional exercise.

### **3.3.2 Diet**

FiberEzy® and timothy haylage were the two products offered as feed during this trial. FiberEzy® is a combination of ensiled lucerne and timothy grass (*Phleum pratense*) (Fibre Fresh, Reporoa, New Zealand). Timothy grass (*Phleum pratense*) was harvested from a single field, ensiled and stored in bags. Both feeds were stored in bags weighing approx. 22.7 kg which were stored on pallets on site.

### **3.3.3 Environment**

The loose boxes consisted of half walls and upper wall wire mesh so horses could observe each other in the trial but not physically interact. The feeds were offered in feed plastic buckets which were placed in the left back corner of each pen. Water was available *ad libitum* as there were automatic drinkers available in each pen on the right back corner.

### **3.3.4 Experimental design**

Horses were randomly paired and assigned to be fed FiberEzy® or timothy for two 18 day periods, in a 2 x 2 Latin square experimental design. Each 18 day period comprised of a 14 day dietary adaptation period where the horses were habituated to the pens and diet, and a 4 d (96 h) total faecal collection (Ordakowski et al., 2001). The horses were fed at 2.5% DM of their initial BW, with the daily allowance divided equally into two meals and fed at 0800 h and 2000 h (Ordakowski-Burk et al., 2006). The feed was weighed before offering to the horses, and all previously unconsumed feed was removed and weighed.

### **3.3.5 Collection of samples**

After the 14 d acclimation period, total collection of faeces was carried out for 4 d using methodology adapted from Ordakowski et al. (2001). Total daily faecal output was calculated by collection of fresh faeces. The faeces were collected into large

plastic containers that were kept closed to maintain the moisture content of the faeces and to prevent any contamination. The complete 12 h collection samples were weighed, a subsample was collected and the remaining faeces were discarded. The subsamples were collected at every 12 h period (1800 gm) to 96 h (400gm). Each time the quantity of the subsample collected was decreased by 200 gm. Each subsample was labelled and frozen at -20°C until freeze-drying at a later date. A sample of the feeds was collected for lab analysis. Subsamples of faeces were freeze-dried and ground through a 0.5 mm screen in a Riechert Mill. Each freeze dried faecal subsample was weighed out into sample bottles to make up a total of 16 gm, from the 12 h sample to 96 h sample all pooled together. Once the samples were weighed out in bottles, the 12 h sample was submitted to the Nutrition Laboratory for analysis. The other subsamples were stored for later analysis if required.

### **3.3.6 Chemical analysis**

The feed and faecal analyses for DM and GE were performed on freeze dried samples which were obtained during the trials. The GE of the feed represents the amount of heat produced from the total combustion of that feed, as measured in a bomb calorimeter. Dry matter was determined by dividing the weight of the hay or faeces after drying by the wet weight of the hay or faeces as sampled. Apparent DM digestibility was calculated using the mean daily DMI and the mean daily faecal DM output (FO) using the equation:

$$\text{DM digestibility} = (\text{DMI} - \text{FO})/\text{DMI} \text{ (Ordakowski-Burk et al., 2006)}$$

## **3.4 Results**

There appeared to be individual horse variation in apparent digestibility of FiberEzy® and timothy haylage. Individual variation was also seen between horses and between groups of horses on the two diets over the experimentation period.

Table 3.1 Shows the Digestible energy content DM (MJ/kg) for FiberEzy® and timothy and Table 3.2 shows the digestibility of each horse on each diet.

**Table 3.1:** Digestible energy content DM (MJ/kg) for FiberEzy® and timothy.

	1 <sup>st</sup> half of study	2 <sup>nd</sup> half of study	Average
FiberEzy®	9.69	9.23	9.51
Timothy	6.88	9.37	8.13

**Table 3.2:** Apparent digestibility of feed by each horse.

		First half of the trial			
Feed	Horse	Total Energy Intake (MJ/d)	Total Energy Excreted (MJ/d)	Apparent Digestibility of the Energy	DE Content DM (MJ/kg)
Timothy	A	142.4	86.2	0.395	7.51
Timothy	B	156.4	104.9	0.329	6.30
FiberEzy®	C	180.7	88.1	0.512	9.90
FiberEzy®	D	180.6	92.3	0.489	9.50
		Second half of the trial			
Feed		Total Energy Intake (MJ/d)	Total Energy Excreted (MJ/d)	Apparent Digestibility of the Energy	DE Content DM (MJ/kg)
FiberEzy®	A	165.1	86.4	0.477	9.10
FiberEzy®	B	168.7	88.6	0.475	9.10
Timothy	C	93.7	44.1	0.530	10.20
Timothy	E	130.5	72.7	0.443	8.50

Apparent DM digestibility was greater for horses consuming FiberEzy® compared with horses fed timothy, except Horse C who consumed less feed over all but has high DE content of timothy compared to all the other horses. Horse D had to be replaced for the 2<sup>nd</sup> half of the digestibility trial due to digestive disturbances (diarrhoea) and reduced feed consumption which led to weight loss; he was replaced by another horse.

### 3.5 Discussion

This study compared two ensiled forages for horses. The comparison was done to gain an insight into its feeding value and to identify whether there were any differences in the digestibility of the forages. The results showed variation in the digestibility of timothy haylage and FiberEzy® and a difference in the consumption of the forage between groups of horses as well. On the basis of feed intake FiberEzy® was preferred compared to timothy haylage. One of the horses on the trial reduced feed intake when changed from FiberEzy® to timothy, resulting in diarrhoea and weight loss, and subsequent removal of this horse from the study. There was a difference in the consumption and digestibility of the two feeds amongst the same group of horses as well. The difference in digestibility may result in less material leaving the gut, thus increasing the bulk fill (Ordakowski-Burk et al., 2006). However, lower digestibility could be associated with an increased mean retention time (Drogoul et al., 2000; Medina et al., 2002).

Dry matter digestibility for forage is an important attribute to consider when feeding horses because it reflects the nutritive value of the forage and helps predict the performance of the animal (Ordakowski-Burk et al., 2006). Nutrient digestibility of forages fed to horses is influenced by several factors including nutrient content and plant maturity, the latter being positively correlated to fibre content (Darlington et al., 1968). Moore-Colyer and Longland (2000) found that clamp silage, bale silage, and haylage were readily digested by ponies, whereas the apparent digestibility of hay was lower than that of fermented forages and marginally lower than other published values for a variety of grass hays fed to horses. It was suggested that these differences probably reflect the variation in species, soil conditions and cutting time of each type of hays. Ragnarsson and Lindberg (2008) reported that early cut timothy haylage (stem elongation to flowering) has the necessary energy content to form the basis of diets for high performance horses. They also reported that late-cut timothy haylage (seeding to late seeding) could be used to fulfil the energy and protein requirement for maintenance-fed Icelandic horses. The influence of the cutting time on the nutritive value and digestibility of timothy in ruminants (Lindberg, 1988) can be explained by

changes in chemical composition of the fibre fraction with advancing plant maturity (Van Soest, 1994).

The energy content of timothy varied considerably between the first and second half of the study, and one horse was removed from the study after changing from FiberEzy® to timothy, meaning the apparent digestibility results should be interpreted with caution. The considerable variation in energy content of timothy could have been due to differences in the stage of maturity at harvesting. The development of diarrhoea by one horse when changed from FiberEzy® to timothy was not investigated to definitively determine the cause of the diarrhoea, nor if the horse had any underlying gastrointestinal problems.

### 3.6 References

- AOAC. (1975). *Official Method of Analyses* (12th ed.). Washington, DC: Association of Official Analytical Chemists.
- Bergero, D., & Peiretti, P. G. (2011). Intake and Apparent Digestibility of Permanent Meadow Hay and Haylage in Ponies. *Journal of Equine Veterinary Science*, 31(2), 67-71. doi: 10.1016/j.jevs.2010.12.006
- Crozier, J. A., Allen, V. G., Jack, N. E., Fontenot, J. P., & Cochran, M. A. (1997). Digestibility, apparent mineral absorption, and voluntary intake by horses fed alfalfa, tall fescue, and caucasian bluestem. *Journal of Animal Science*, 75(6), 1651-1658.
- Cunha, T. J. (1980). *Horse feeding and nutrition*: Academic Press, Inc.(London) Ltd.
- Darlington, J. M., & Hershberger, T. V. (1968). Effect of Forage Maturity on Digestibility, Intake and Nutritive Value of Alfalfa, Timothy and Orchardgrass by Equine. *Journal of Animal Science*, 27(6), 1572-1576.
- Divers, T. J., Bartholomew, R. C., Messick, J. B., Whitlock, R. H., & Sweeney, R. W. (1986). CLOSTRIDIUM-BOTULINUM TYPE-B TOXICOSIS IN A HERD OF CATTLE AND A GROUP OF MULES. [Article]. *Journal of the American Veterinary Medical Association*, 188(4), 382-386.
- Dodds, K. L. (1994). *Clostridium botulinum*. In: *Handbook of Foodborne Diseases*. USA: Marcel Decker Inc.
- Drogoul, C., Poncet, C., & Tisserand, J. L. (2000). Feeding ground and pelleted hay rather than chopped hay to ponies: 1. Consequences for in vivo digestibility and rate of passage of digesta. *Animal Feed Science and Technology*, 87(1-2), 117-130. doi: [http://dx.doi.org/10.1016/S0377-8401\(00\)00187-5](http://dx.doi.org/10.1016/S0377-8401(00)00187-5)
- Frape, D. (2004). *Equine Nutrition & Feeding* (3rd ed.). Oxford: Blackwell Publishing Ltd.
- Hailu, K., Bettey, R. L., Ardans, A., Galey, F. D., Daft, B. M., Walker, R. L., . . . Byrd, J. W. (1991). Clostridium botulinum type C intoxication associated with consumption of processed alfalfa hay cube in horses. *J. Am. vet. med. Ass.*, 199, 742-746.
- Henneke, D. R., Potter, G. D., Kreider, J. L., & Yeates, B. F. (1983). Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Veterinary Journal*, 15(4), 371-372. doi: 10.1111/j.2042-3306.1983.tb01826.x
- Jonsson, A. (1989). The role of yeasts and Clostridia in silage deteriorations. *Rapport nr. 42. Sveriges Lantbruksuniversitet*, 11-22. .
- Lewis, L. D. (1995). *Equine Clinical Nutrition : Feeding and Care*. PA, USA: Williams & Wilkins.
- Lindberg, J. (1988). Influence of cutting time and N-fertilization on the nutritive value of timothy, 2: Estimates of rumen degradability of nitrogenous compounds. *Swedish Journal of Agricultural Research*, 18.
- McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., & Morgan, C. A. (2002). *Animal nutrition* (6th ed.). Harlow: UK Prentice Hall.
- Medina, B., Drogoul, C., & Julliand, V. (2002). *Feeding practices and digestive transit*. Paper presented at the 28ème journée de la recherche équine 27 février 2002.
- Moore-Colyer, M. J. S., & Longland, A. C. (2000). Intakes and in vivo apparent digestibilities of four types of conserved grass forage by ponies. *Journal of Animal Science*, 71, 527-534. doi: 1357-7298/00/07890527

- Müller, C. E. (2005). Fermentation patterns of small-bale silage and haylage produced as a feed for horses. *Grass and Forage Science*, 60(2), 109-118. doi: 10.1111/j.1365-2494.2005.00457.x
- Müller, C. E., & Udén, P. (2007). Preference of horses for grass conserved as hay, haylage or silage. *Animal Feed Science and Technology*, 132(1–2), 66-78. doi: 10.1016/j.anifeedsci.2006.02.013
- Notermans, S., Kozaki, S., & Vanschothorst, M. (1979). Toxin production by *Clostridium botulinum* in grass. *Applied and Environmental Microbiology*, 38(5), 767-771.
- NRC. (1989) *Nutrient Requirements of Horses* (6th ed.). Washington DC . The National Academy Press.
- NRC. (2007) *Nutrient Requirement of Horses* (6th ed.). Washington, DC: The National Academies Press.
- Ordakowski-Burk, A. L., Quinn, R. W., Shellem, T. A., & Vough, L. R. (2006). Voluntary intake and digestibility of reed canarygrass and timothy hay fed to horses. *Journal of Animal Science*, 84(11), 3104-3109. doi: 10.2527/jas.2005-607
- Ordakowski, A. L., Kronfeld, D. S., Holland, J. L., Hargreaves, B. J., Gay, L. S., Harris, P. A. & Sklan, D. (2001). Alkanes as internal markers to estimate digestibility of hay or hay plus concentrate diets in horses. *Journal of Animal Science*, 79(6), 1516-1522.
- Ragnarsson, S., & Lindberg, J. E. (2008). Nutritional value of timothy haylage in Icelandic horses. 113(2), 202-208.
- Small, E. (1996). Adaptations to herbivory in alfalfa (*Medicago sativa*). *Canadian Journal of Botany*, 74(6), 807-822.
- Staniar, W. B., Bussard, J. R., Repard, N. M., Hall, M. H., & Burk, A. O. (2010). Voluntary intake and digestibility of teff hay fed to horses. *Journal of Animal Science*, 88(10), 3296-3303. doi: 10.2527/jas.2009-2668
- Van Soest, P. J. (1994). *Nutritional ecology of the ruminant*: Cornell University Press.
- Vandenput, S., Istasse, L., Nicks, B., & Lekeux, P. (1997). Airborne dust and aeroallergen concentrations in different sources of feed and bedding for horses. *Veterinary quarterly*, 19(4), 154-158.
- Wilkinson, J. M. (2005). *Silage*. Lincoln, United Kingdom: Chalcombe.

## **CHAPTER FOUR – Voluntary feed intake and Behavioural observation of Thoroughbred horses fed FiberEzy® and Rye Clover hay**

### **4.1 Abstract**

**AIM:** To determine the voluntary feed intake and behavioural observation of six Thoroughbred horses being fed FiberEzy® and Rye clover hay.

**METHODS:** Six Thoroughbred geldings, ( $10 \pm 1$  yr.;  $550.6 \pm 15.8$  kg) were offered FiberEzy® (FE) or Rye-clover hay (RCH) at  $>3\%$  of the body weight. The horses were stalled individually in loose boxes (4 x 4 m) lined with rubber matting. Voluntary feed intake was measured and behaviour monitored over 17 days from days 9-17 of the study during which the horses were scan sampled and videoed for two hours every morning (9:30-11:30 am) after feeding and two hours in the evening (4:30-6:30 pm) after feeding.

**RESULTS:** There were significant differences between time periods ( $24.3 \pm 0.9$  vs  $17.8 \pm 0.4$  kg DM /day,  $p < 0.05$ ) and feeds for VFI (FE  $24.3 \pm 0.4$  vs. RCH  $17.8 \pm 0.7$  kg DM /day) but not an interaction. Behaviours were typical of loose box housed horses and differences in feeding behaviour observed correlated with the differences in VFI measured between feeds.

**CONCLUSION:** There was greater VFI on the FiberEzy® diet and this was associated with a greater eating behaviour observed in the horses. The ensiled product FiberEzy® appears a suitable feed based on VFI and behaviour for feeding to horses.

## 4.2 Introduction

Horses have evolved as grass eaters but, in temperate countries, pasture grass cannot be provided all year due to the cold climate. The grass therefore has to be conserved and traditionally hay-making has been the most common form of conservation. In recent years, silage and haylage have been introduced to equine diets (Müller & Udén, 2007). Forages preserved by ensiling are usually harvested at an earlier stage of maturity than forages preserved as hay. This results in an increase in the digestibility of nutrients and the energy value is markedly increased (Ragnarsson et al., 2008). As a consequence, less dry matter (DM) has to be fed to meet the energy requirements of horses (Ragnarsson et al., 2010b). Although many opinions about the suitability of different conservation methods of grass to be used for horses exist, the scientific knowledge in the area is scarce, and reported work has mainly dealt with voluntary intake and digestibility effects on behaviour or effect of forage species on voluntary intake and digestibility (LaCasha et al., 1999; Müller & Udén, 2007). The nutritive value and digestibility of forages are important parameters, but only if the forage is not rejected by horses. Ensiling has been reported to reduce the voluntary intake of forages in other animal species and in horses (Moore-Colyer et al., 2000), and horse owners sometimes claim that silage will not be eaten by their horses. However, published comparisons including observations of voluntary intake (Moore-Colyer et al., 2000; Moore-Colyer et al., 2003) have included forages with very different nutrient composition.

FiberEzy® is a commercial marketed ensiled product sold as a suitable forage for horses. It is marketed as a balanced safe multiple-fibre feed suitable for horses resting, in convalescence or at risk of nutrition related, metabolic disorders. Perennial ryegrass (*Lolium perenne* L.), also called English ryegrass, is a cool-season perennial bunchgrass native to Europe, temperate Asia, and North Africa. It is widely distributed throughout the world, including North and South America, Europe, New Zealand, and Australia. Ryegrass pastures have high nutrient value and produce excellent animal gains. Ryegrass has higher total nutrients than oats (Hannaway et al., 1999). Excess pasture growth sometimes is cut for hay. Ryegrass can make good hay if it is allowed sufficient

time to dry prior to baling. Ryegrass hay, however, is generally considered to be low in quality and palatability. Red clover hay is a good feed for horses. Clover is a legume, high in protein, some-what laxative in effect. Red clover may be grazed, harvested as green-chop, or made into hay. Red clover grown in pure stands or in combination with a small grain or ryegrass may be used to provide high-quality grazing.

The voluntary feed intake (VFI) of ensiled forages by horses is largely influenced by forage type and the DM content. Horses tend to have a larger intake of high DM (40-65% DM) ensiled forages than low DM (<30% DM) ensiled forages (Cuddeford, 1996; NRC, 2007).

### **4.3 Materials and methods**

#### **4.3.1 Animals**

Six mixed age Thoroughbred geldings, ( $10 \pm 1$  yr.;  $550.6 \pm 15.8$  kg), housed at the LWT Equine Research Unit (Kiwitea, New Zealand), were used for the experiment. Immediately prior to start of the trial the horses had a basic clinical examination which included heart rate and respiration rate. A faecal egg count was done on the six horses. If required the hooves were trimmed by a farrier. Horses were weighed using a live-stock platform scale (Tru-test, ezweigh2) at weekly intervals and a body condition score was assigned after Henneke et al., (1983).

#### **4.3.2 Diets**

The two feeds used in this experiment were Rye-clover hay and FibreEzy. The Rye-clover hay offered was a mixture of red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). The hay was harvested from a single paddock; field dried and baled into approximately 15-15.8 kg conventional bales. After harvest the hay was stored in the shed. The FibreEzy was as described in section of diets in digestibility.

### **4.3.3 Environment**

The loose boxes consisted of half walls and an upper wall of wire mesh so horses could observe each other in the trial but not physically interact. The FiberEzy® was offered in feed plastic buckets which were placed in the left back corner of each pen, whereas, the Rye clover hay was fed in hay nets attached to the grill in the front. Water was available *ad libitum* as there were automatic drinkers available in each pen on the right back corner.

### **4.3.4 Experimental design**

Using a Latin square design the horses were randomly divided into two groups of three and allocated one of the two diets (FiberEzy® (FE) rye clover hay (RCH)). At the start of the trial one group of horses received FE and the second group were fed RCH over a 17 d period after which the feeds were changed to create a cross over design. The horses were kept in rubber matted loose box stalls (4m x 4m) and offered the feed in the morning (0800h) and evening (2000h). The stalls were cleaned twice daily. The horses were given free exercise, individually or in pairs, on the limestone pressed yard outside the stalls (24m by 8m approx.) for 30 – 60 minutes in the morning before the offering of the feed at 0800 h (morning) and 2000 h (evening).

The horses were offered feed to meet their estimated daily maintenance requirements of 2.5% dry matter of initial body weight during the 8 day adaptation period. During the 9 day voluntary DM intake (DMI) period the feed was fed in excess of voluntary intake (~3.2% of BW) (Staniar et al., 2010). The amount of feed consumed and refused was recorded twice daily. The refusals from the evening feed were weighed and subtracted from the value of feed offered in the morning, with the same was done for the refusal found in the morning i.e. it was subtracted from the value of offered evening feed.

The behavioural observations were included as part of the voluntary feed intake experiment to examine if there was a difference in behaviour and feeding when the

horses are offered two different diets, and to examine if there was a difference in feeding behaviour during the adaptation phase and experimental phase.

#### **4.3.5 Behavioural measurement**

The behavioural measurements were obtained using scan sampling (Cooper et al., 2005) and videoing (McDonnell et al., 1990). Scan sampling was done once in the morning after the feed was provided (9:30-11:30 am) and in the evening (4:30-6:30 pm) before feeding to account for diurnal variation in behaviour (Thorne et al., 2005), simultaneously the horses were videoed to observe behaviour during the 17 day period i.e. the initial four days of the adaptation and the last four days of the 9d VFI period to see whether there was a difference in the quantity of feed consumed, time required by the horses to adapt from one feed to the other over the adaptation phase, as well as, if there was a change in the eating period bout from one diet to another. Differences in feeding during the adaptation phase and the voluntary feed intake period was observed a whole, as well as if there was a difference between horses on the same and different feed. The camera was placed on the right front corner outside the pen at an angle that permitted viewing of the horse. For scan sampling each horse was scanned once every 2 mins (61 scans per observation period) and on each scan the behaviour was recorded (Cooper et al., 2005; Ninomiya et al., 2007). The ethogram used included categories such as general activity, feeding and other ingestive behaviour, stereotypic behaviour and other activities defined in table 4.1.

Behaviour recording aimed to capture the spectrum of equine activity expected to be observed in loose box confined horses.

**Table 4.1:** Ethogram of stereotypic behaviour measured incorporated from Cooper et al. (2005)

Category	Definition
Stand alert	Rigid stance, with the neck elevated and the head orientated toward the object of focus. The ears are held stiffly upright and forward and the nostrils might be slightly dilated (McDonnell et al., 1995)
Stand doze	Standing but not appearing to pay attention to surroundings. Typically eyes shut or half-closed, ears not erect (Cooper, et al., 2000)
Locomotion	Moving from one location in pen to another. Not overtly repetitive (see Box walking)
Eating	Horse is actively chewing and swallowing food/edible material (McAfee et al., 2002)
Drink	Drinking from automatic drinker
Licking	Licking the grill or wood
Nod	Repetitive vertical movement of head
Oral stereotypy	Repetitive oral activities without overt nutritional function, such as sham chewing, tongue rolling, biting or grasping stable fittings or repeated licking of pen fittings (McGreevy, PD et al., 2010)
Box walking	Repeatedly moving between two locations in pen or walking a route around the stable (Cooper, et al., 2005)
Head rubbing	Horse is rubbing its head on some physical object

Rubbing	Rubbing of any body parts on the walls of the stable (Clegg et al., 2008)
Door kick/paw	Kicking pen door (or other fittings), stamping or scraping foot on stable floor (Christensen et al., 2005; Cooper, et al., 2005)
Other	Any activity not covered by the above (e.g. urination)

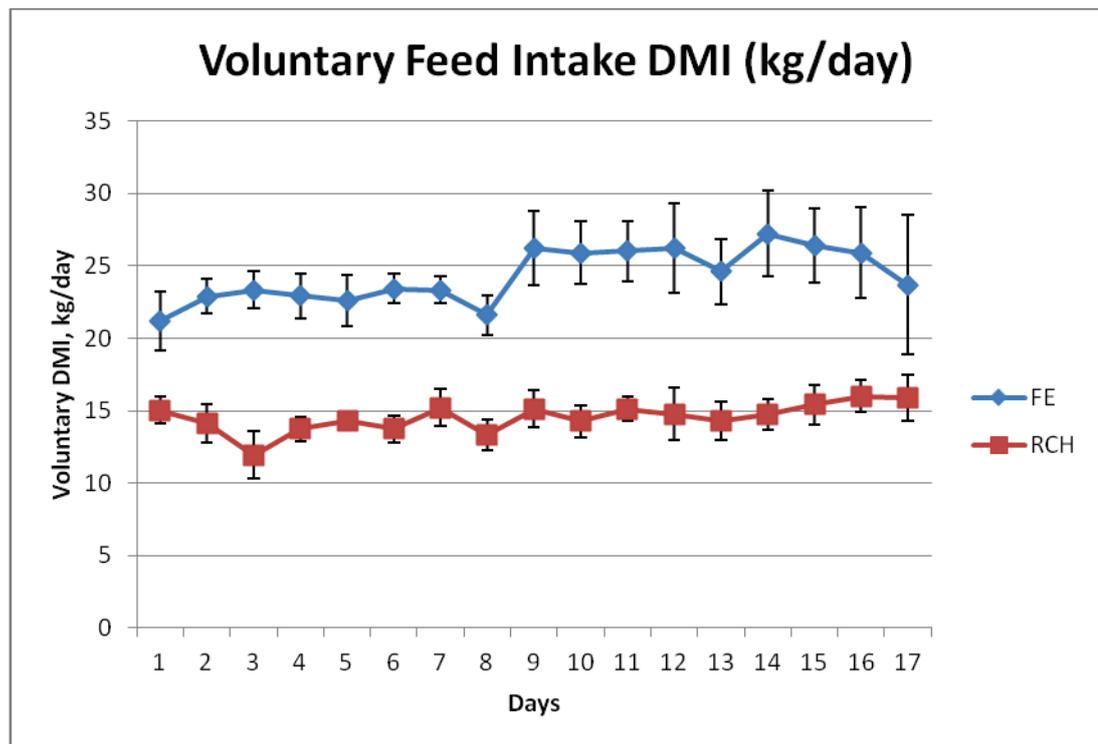
#### 4.3.7 Behaviour analyses

Data were transcribed to MS Excel (Microsoft Excel 2010) and summary tables generated.

#### 4.4 Results

There were significant differences in the VFI of feeds during the VFI study. There was a significant block effect with horses consuming more feed during the first block of the trial ( $24.3 \pm 0.9$  vs  $17.8 \pm 0.4$  kg DM /day,  $p < 0.05$ ). There was no significant effect of group on VFI ( $20.3 \pm 0.4$  vs  $21.8 \pm 0.7$  kg DM /day). There were significant differences in the VFI between feeds with greater quantities of FE consumed relative to RCH ( $24.3 \pm 0.4$  vs.  $17.8 \pm 0.7$  kg DM /day). There was some individual horse variation and variation over time (Figure 4.1).

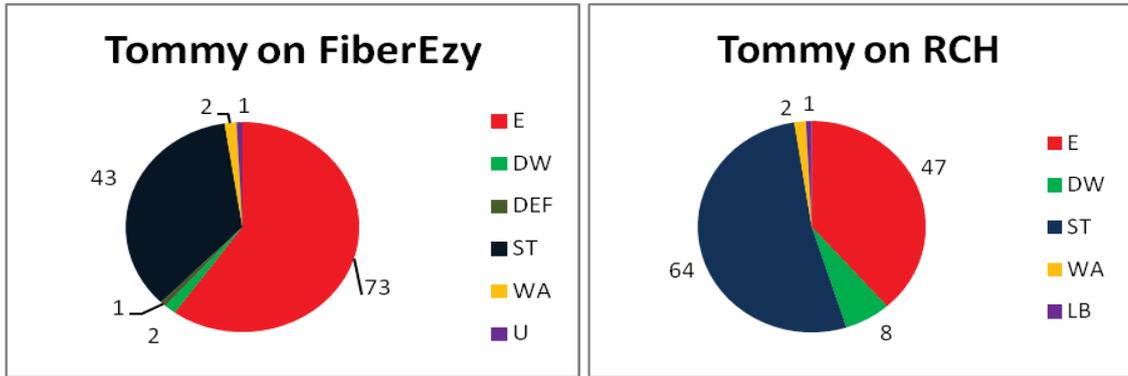
**Figure 4.1:** Average voluntary DMI (kg/d) by Thoroughbred geldings fed on FiberEzy® (FE; n =6) or Rye Clover hay (RCH; n=6)



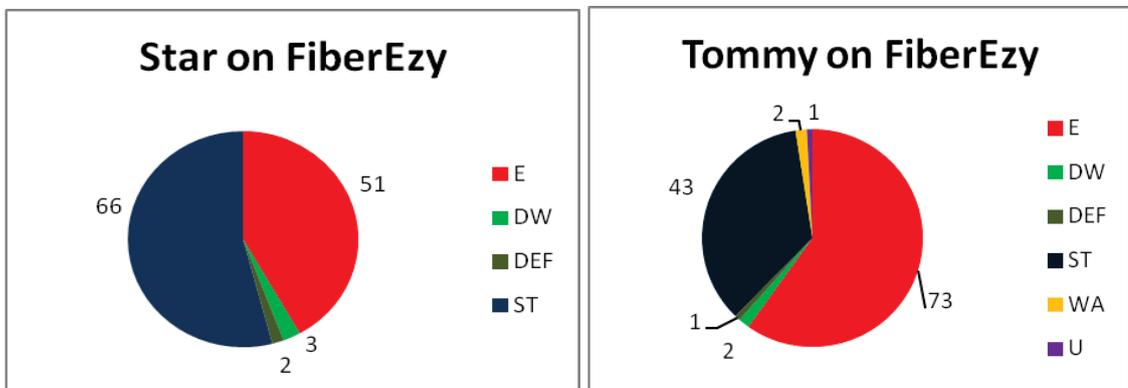
#### 4.4.1 Behaviour Activity:

The horses were observed for the various other behaviours observed during the exercise period when they were let out in the yard outside the pens. The horses were seen to groom each other, play around, roll, as well as, stand and just observe the surroundings. Abnormal stereotypic behaviour was seen in Boots, who would wind suck when was let out for the exercise period. All the other horses were seen to have normal behaviour in the pens, except for occasional excitability where Robin would kick the box in response to behaviour shown by Tommy during the VFI trial, as well as, they would make noises as the feed was taken to them or would paw at the door.

**Figure 4.2:** A representation of the behaviour of a single horse on two different feeds

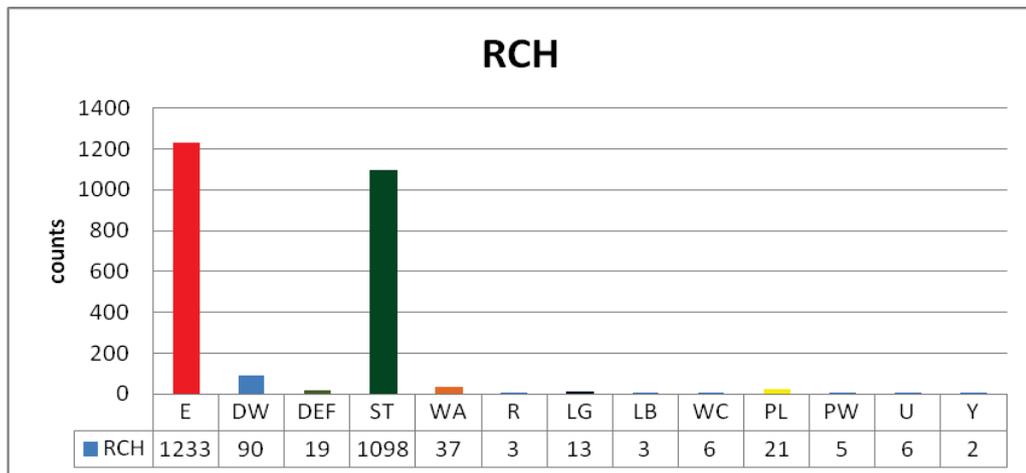
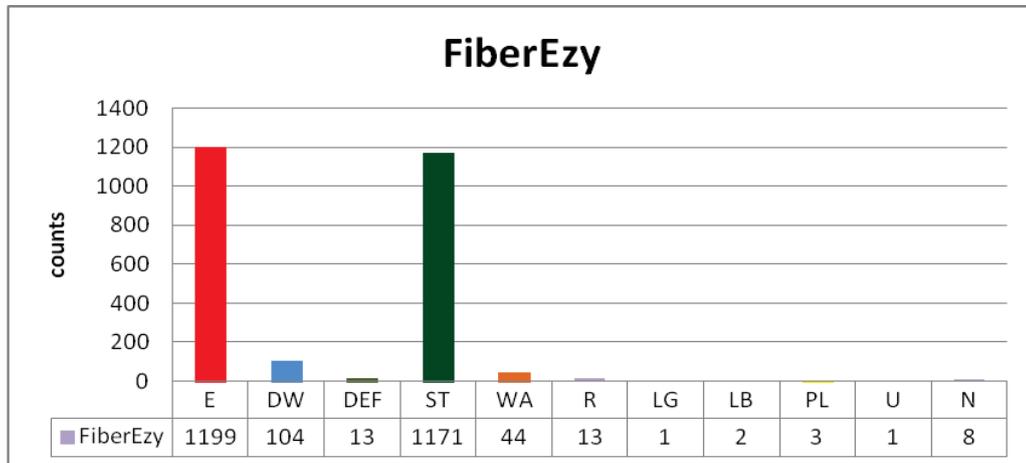


**Figure 4.3:** A representation of the behaviour of two different horses on a single feed



Scan behavioural observations done every 2 mins shows the activity of the horses during those two hours of the day and can correlate it to the activity monitor reading, as to what the horse could be doing at that particular timing. But the foot movement cannot be predictable that well as it was noticed the horse on FiberEzy® would stand in one place eating for a long time, whereas the horse eating rye clover hay would walk around more either to drink water or just have a stroll. It can also be seen in Figure 4.2 and Figure 4.3 that Tommy showed variation in behaviour on two different feeds.

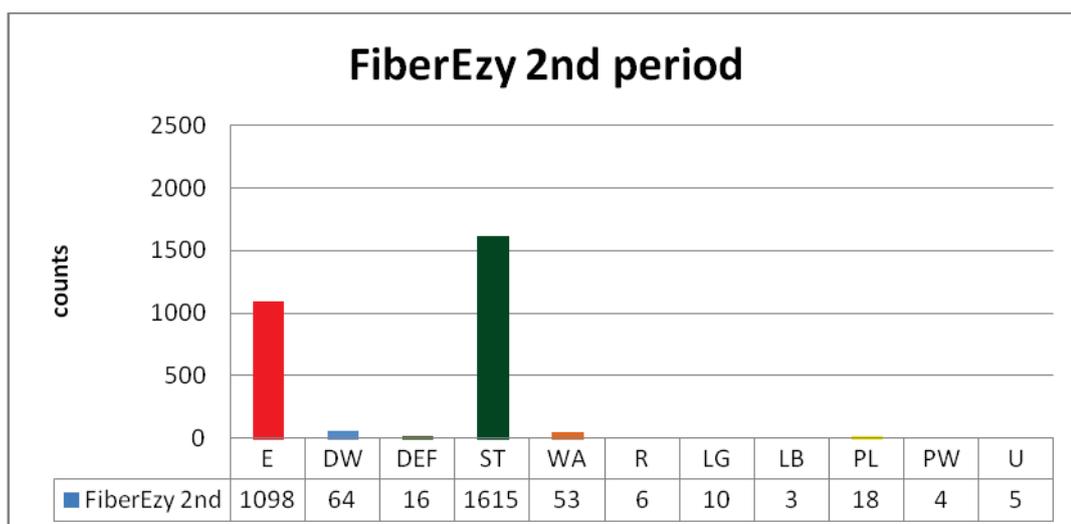
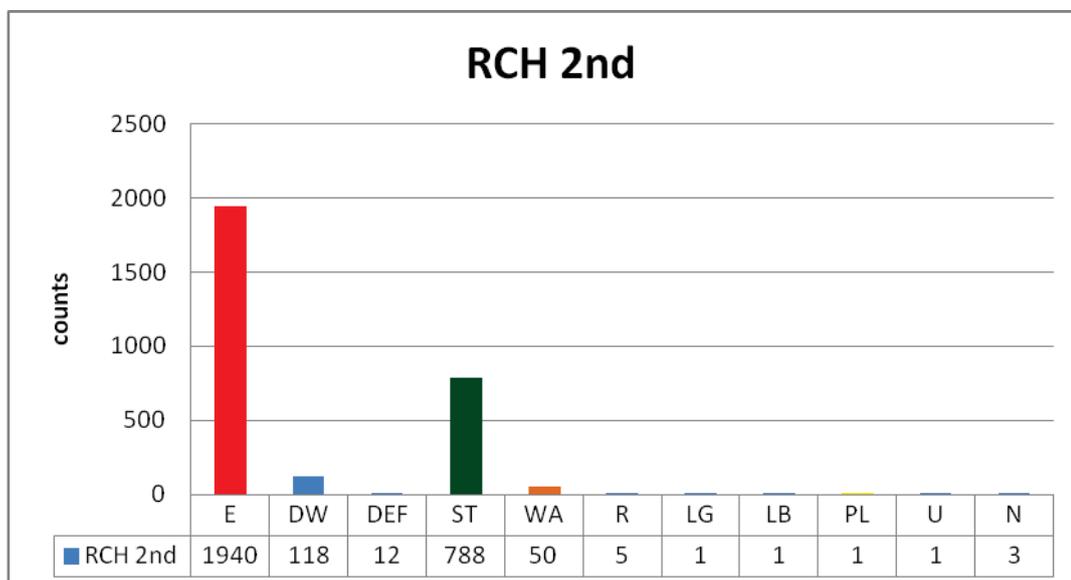
**Figure 4.3:** Collective behaviour shown by the two group of horses (n=4) on two different feeds i.e. FiberEzy® and Rye Clover Hay during the first Voluntary Feed Intake period



List of abbreviations used for the bar graphs

<b>E = eating</b>	<b>LB = licking water bowl</b>
<b>DW = drinking</b>	<b>BG = biting grill</b>
<b>DEF = defecating</b>	<b>WC = wood chewing</b>
<b>ST = standing</b>	<b>PL = playing with each other</b>
<b>WA = wandering</b>	<b>PW = pawing</b>
<b>R = rubbing</b>	<b>U = urinating</b>
<b>LG = licking grill</b>	<b>C = chewing</b>

**Figure 4.4:** Collective behaviour shown by the two group of horses (n=4) on two different feeds i.e. FiberEzy® and Rye Clover Hay during the second Voluntary Feed Intake period



List of abbreviations used for the bar graphs

<b>E = eating</b>	<b>LB = licking water bowl</b>
<b>DW = drinking</b>	<b>BG = biting grill</b>
<b>DEF = defecating</b>	<b>WC = wood chewing</b>
<b>ST = standing</b>	<b>PL = playing with each other</b>
<b>WA = wandering</b>	<b>PW = pawing</b>
<b>R = rubbing</b>	<b>P = peeing</b>
<b>LG = licking grill</b>	<b>C = chewing</b>

A variation in the feeding behaviour can be observed looking at the graph for the same set of horses on different feed after the crossover of feeds during the VFI trial. The group of horses feeding on RCH are seen spending more time eating then standing compared to the horses feed FiberEzy® where they apparently are taking a less time feeding and are seen to spend more time standing. It has even been observed horses eating hay drink more water.

#### **4.5 Discussion**

Voluntary DMI of forage is a function of its chemical, physical, and morphological characteristics as well as its digestibility(Ordakowski-Burk et al., 2006; Van Soest, 1965). The comparison of nutrient composition of the FiberEzy® and RCH was determined to be significantly different. . Mean voluntary DMI intake of FiberEzy® was greater than RCH in our study over the 14-day experimental period, differences were most pronounced during the first week on day 3 and 6 (figure 4.1) and day 14 of week 2. Two weeks of collection for the voluntary DMI was used to ensure that data collected would be a good representative sample of intake. There was a difference in the voluntary DMI of each the feed as seen in figure 4.1, whereby DMI was greater for FE compared to RCH.

The behaviour observations was based on the 2 min scan sampling , activity monitor reading from the head and foot movement as well as video observation during the two hour scan sampling period. Large variation was seen in the behaviour of individual horses before and after meals as well as when they were left out in the paddock during the cleaning of the pens. There was a difference seen in the eating bouts for the horses on the two different feeds. The horses were seen to show natural behaviour when they were left in the open paddock. During the scan sampling the horses were shown to be more active in the morning after the feed was given to them and the similar active behaviour was observed before the feeding. One horse was seen to paw as soon as he knew he was going to be fed and they would grunt. Aggression was seen in some horses during the trial such as kicking the boxes and playing with each other through the grill.

The behaviours observed were typical of horse maintained in loose boxes and there was some between-horse variation. The differences in behaviour observed between the two feeds reflected the differences observed in the VFI and the assumed palatability of the two feeds.

## 4.6 References

- AgReaserch. Timothy-Grasslanz Unique plant opportunities Retrieved 24/10/2012, from <http://www.grasslanz.com/LinkClick.aspx?fileticket=b5OXDbWHCVs%3D&tabid=78>
- Altmann, J. (1974). Observational Study of Behavior: Sampling Methods. *Behaviour*, 49(3/4), 227-267. doi: 10.2307/4533591
- Archer, M. (1971). Preliminary studies on the palatability of grasses, legumes and herbs to horses. *Veterinary Record*, 89(9), 236-240.
- Archer, M. (1978). Studies on Producing and Maintaining Balanced Pastures for Studs. *Equine Veterinary Journal*, 10(1), 54-59. doi: 10.1111/j.2042-3306.1978.tb02216.x
- Baalsrud, K. J., & Overnes, G. (1986). Influence of vitamin E and selenium supplement on antibody production in horses. *Equine Veterinary Journal*, 18(6), 472-474.
- Bachmann, I., Audigé, L., & Stauffacher, M. (2003). Risk factors associated with behavioural disorders of crib-biting, weaving and box-walking in Swiss horses. *Equine Veterinary Journal*, 35(2), 158-163. doi: 10.2746/042516403776114216
- Bagshaw, C., Ralston, S., & Fisher, H. (1994). Behavioral and physiological effect of orally administered tryptophan on horses subjected to acute isolation stress. *Applied Animal Behaviour Science*, 40(1), 1-12.
- Ballet, N., Robert, J., & Williams, P. (2000). 19 Vitamins in Forages. *Forage Evaluation in Ruminant Nutrition*, 399.
- Battle, G. H., Jackson, S., & Baker, J. (1988). Acceptability and digestibility of preservative-treated hay by horses. *Nutrition reports international*, 37(1), 83-89.
- Bels, V. L. (2006). *Feeding in domestic vertebrates : from structure to behaviour / edited by V. Bels*: Cambridge, MA : CABI Pub., c2006.
- Berg, C. C., McElroy, A. R., & Kunelius, H. T. (1996a). Timothy. *Cool-season forage grasses(coolseasonforag)*, 643-664.
- Berg, C. C., McElroy, A. R., & Kunelius, H. T. (1996b). Timothy1. *Cool-season forage grasses, agronomyonogra(coolseasonforag)*, 643-664. doi: 10.2134/agronmonogr34.c20
- Bergero, D., & Peiretti, P. G. (2011). Intake and Apparent Digestibility of Permanent Meadow Hay and Haylage in Ponies. *Journal of Equine Veterinary Science*, 31(2), 67-71. doi: 10.1016/j.jevs.2010.12.006
- Bergero, D., Peiretti, P. G., & Cola, E. (2002). Intake and apparent digestibility of perennial ryegrass haylages fed to ponies either at maintenance or at work. *Livestock Production Science*, 77(2-3), 325-329. doi: 10.1016/s0301-6226(02)00031-3
- Blakeslee, J. K. (1974). *Mother-young relationships and related behavior among free-ranging Appaloosa horses*: Idaho State University.
- Boyd, L. E., Carbonaro, D. A., & Houpt, K. A. (1988). The 24-hour time budget of Przewalski horses. *Applied Animal Behaviour Science*, 21(1), 5-17.
- Brown, F. (1953). The tocopherol content of farm feeding-stuffs. *Journal of the Science of Food and Agriculture*, 4(4), 161-165.
- Buchanan-Smith, J. (1990). An investigation into palatability as a factor responsible for reduced intake of silage by sheep. *Anim. Prod*, 50, 253-260.

- Burk, A. O., & Williams, C. A. (2008). Feeding management practices and supplement use in top-level event horses. *Comparative Exercise Physiology*, 5(02), 85-93. doi:doi:10.1017/S1478061508062786
- Cameron, E. Z., Stafford, K. J., Linklater, W. L., & Veltman, C. J. (1999). Suckling behaviour does not measure milk intake in horses, *Equus caballus*. *Animal Behaviour*, 57(3), 673-678. doi: <http://dx.doi.org/10.1006/anbe.1998.0997>
- Christensen, J. W., Keeling, L. J., & Nielsen, B. L. (2005). Responses of horses to novel visual, olfactory and auditory stimuli. *Applied Animal Behaviour Science*, 93(1-2), 53-65. doi: 10.1016/j.applanim.2005.06.017
- Christie, J. L., Hewson, C. J., Riley, C. B., McNiven, M. A., Dohoo, I. R., & Bate, L. A. (2006). Management factors affecting stereotypes and body condition score in nonracing horses in Prince Edward Island. *Canadian Veterinary Journal*, 47(2), 136-143.
- Chung, T. K., & Baker, D. H. (1992). Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. *Journal of Animal Science*, 70(12), 3781-3790.
- Clegg, H. A., Buckley, P., Friend, M. A., & McGreevy, P. D. (2008). The ethological and physiological characteristics of cribbing and weaving horses. *Applied Animal Behaviour Science*, 109(1), 68-76. doi: 10.1016/j.applanim.2007.02.001
- Cooper, J. J., McAll, N., Johnson, S., & Davidson, H. P. B. (2005). The short-term effects of increasing meal frequency on stereotypic behaviour of stabled horses. *Applied Animal Behaviour Science*, 90(3-4), 351-364. doi: 10.1016/j.applanim.2004.08.005
- Cooper, J. J., McDonald, L., & Mills, D. S. (2000). The effect of increasing visual horizons on stereotypic weaving: implications for the social housing of stabled horses. *Applied Animal Behaviour Science*, 69(1), 67-83. doi: 10.1016/s0168-1591(00)00115-5
- Crozier, J. A., Allen, V. G., Jack, N. E., Fontenot, J. P., & Cochran, M. A. (1997). Digestibility, apparent mineral absorption, and voluntary intake by horses fed alfalfa, tall fescue, and caucasian bluestem. *Journal of Animal Science*, 75(6), 1651-1658.
- Cuddeford, D. (1996). *Equine Nutrition*. Ramsbury, Marlborough: The Crowood Press Ltd.
- Cuddeford, D., Garnsworthy, P., & Wiseman, J. (2002). Equine nutrition: some unique features, functions and frailties of the digestive system of the horse. *Recent advances in animal nutrition 2002*, 269-318.
- Cunha, T. J. (1980). *Horse feeding and nutrition*: Academic Press, Inc.(London) Ltd.
- Darlington, J. M., & Hershberger, T. V. (1968). Effect of Forage Maturity on Digestibility, Intake and Nutritive Value of Alfalfa, Timothy and Orchardgrass by Equine. *Journal of Animal Science*, 27(6), 1572-1576.
- Davidson, N., & Harris, P. (2003). Nutrition and Welfare. In N. Waran (Ed.), *The Welfare of Horses* (Vol. 1, pp. 45-76): Springer Netherlands.
- Davis, Z. (2009). *Introduction to Horse Nutrition*: Blackwell Publishing Ltd.
- Divers, T. J., Bartholomew, R. C., Messick, J. B., Whitlock, R. H., & Sweeney, R. W. (1986). CLOSTRIDIUM-BOTULINUM TYPE-B TOXICOSIS IN A HERD OF CATTLE

- AND A GROUP OF MULES. [Article]. *Journal of the American Veterinary Medical Association*, 188(4), 382-386.
- Dodds, K. L. (1994). *Clostridium botulinum*. In: *Handbook of Foodborne Diseases*. USA: Marcel Decker Inc.
- Drogoul, C., Poncet, C., & Tisserand, J. L. (2000). Feeding ground and pelleted hay rather than chopped hay to ponies: 1. Consequences for in vivo digestibility and rate of passage of digesta. *Animal Feed Science and Technology*, 87(1-2), 117-130. doi: [http://dx.doi.org/10.1016/S0377-8401\(00\)00187-5](http://dx.doi.org/10.1016/S0377-8401(00)00187-5)
- Dutton, M. F., Westlake, K., & Anderson, M. S. (1984). The interaction between additives, yeasts and patulin production in silage. *Mycopathologia*, 87(20-33).
- Dyce, K. M., Sack, W. O., & Wensing, C. J. G. (2009). *Textbook of veterinary anatomy*: Saunders.
- Ellis, A., Longland, A., Coenen, M., & Miraglia, N. (2010). Biological basis of behaviour in relation to nutrition and feed intake in horses. *EAAP publication*, 128, 53-74.
- Ellis, A. D., & Hill, J. (2005). *Nutritional physiology of the horse*: Nottingham University Press.
- Erdman, R. (1993). *Silage fermentation characteristics affecting feed intake*. Paper presented at the National Silage Production Conference.
- Evans, J. W. (2000). *Horses - A Guide to Selection, Care and Enjoyment* (3rd ed.): WH Freeman and Company, New York, NY.
- Frame, J., & Harkess, R. D. (1987). The Productivity of 4 forage legumes sown alone and with each of 5 companion grasses. [Article]. *Grass and Forage Science*, 42(3), 213-223. doi: 10.1111/j.1365-2494.1987.tb02109.x
- Frame, J., Harkess, R. D., & Hunt, I. V. (1985). Effect of seed rate of red clover and of companion timothy or tall fescue on herbage production. *Grass and Forage Science*, 40(4), 459-465. doi: 10.1111/j.1365-2494.1985.tb01777.x
- Francis-Smith, K. (1977). Behavior patterns of horses grazing in paddocks. *Appl Anim Ethol*, 3, 292-293.
- Frandsen, R. D., Wilke, W. L., & Fails, A. D. (2009). *Anatomy and physiology of farm animals*: Wiley-Blackwell.
- Frape, D. (2004). *Equine Nutrition & Feeding* (3rd ed.). Oxford: Blackwell Publishing Ltd.
- Gidenne, T. (1992). Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. *British Journal of Nutrition*, 67(01), 133-146.
- Gill, A. M., Parker, A., Leak, S., & Lawrence, L. M. (1998). Effects of meal feeding frequency and roughage on behaviors of stabled horses. *Advances in Equine Nutrition*, 89-92.
- Gillham, S. B., Dodman, N. H., Shuster, L., Kream, R., & Rand, W. (1994). The effect of diet on cribbing behavior and plasma  $\beta$ -endorphin in horses. *Applied Animal Behaviour Science*, 41(3), 147-153.
- Grier, J. W., & Burk, T. (1984). *Biology of animal behavior*: Times Mirror/Mosby College Pub.
- Gudmundsdottir, K. B., Svansson, B., Aalbaek, B., Gunnarsson, E., & Sigurdarson, S. (2004). *Listeria monocytogenes* in horses in Iceland. *Veterinary Record*, 155(456-459).

- Haenlein, G. F. W., Holdren, R. D., & Yoon, Y. M. (1966). Comparative Response of Horses and Sheep to Different Physical Forms of Alfalfa Hay. *Journal of Animal Science*, 25(3), 740-743.
- Hailu, K., Bettey, R. L., Ardans, A., Galey, F. D., Daft, B. M., Walker, R. L., . . . Byrd, J. W. (1991). Clostridium botulinum type C intoxication associated with consumption of processed alfalfa hay cube in horses. *J. Am. vet. med. Ass.*, 199, 742–746.
- Hakkarainen, R. J., Työppönen, J. T., & Bengtsson, S. G. (1983). Relative and quantitative changes in total vitamin E and isomer content of barley during conventional and airtight storage with special reference to annual variations. *Acta Agriculturae Scandinavica*, 33(4), 395-400.
- Hall, M. H., & Cherney, J. H. (1914). Timothy- Agronomy Facts 24. *PennState College of Agriculture-Cooperative Extension*.
- Hannaway, D. B., Fransen, S., Cropper, J. B., Teel, M., Chaney, M., Griggs, T. D., . . . Lane, W. (1999). Perennial ryegrass (*Lolium perenne* L.).
- Henneke, D. R., Potter, G. D., Kreider, J. L., & Yeates, B. F. (1983). Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Veterinary Journal*, 15(4), 371-372. doi: 10.1111/j.2042-3306.1983.tb01826.x
- Hintz, H., & Cymbaluk, N. (1994). Nutrition of the horse. *Annual review of nutrition*, 14(1), 243-267.
- Hoffmann, R. (1985). On the development of social behaviour in immature males of a feral horse population (*Equus przewalskii* f. *caballus*). *Zeitschrift f̃r S̃eugetierkunde*, 50, 302–314.
- Holland, J., Kronfeld, D., & Meacham, T. (1996). Behavior of horses is affected by soy lecithin and corn oil in the diet. *Journal of Animal Science*, 74(6), 1252-1255.
- Horvath, G., Wessjohann, L., Bigirimana, J., Jansen, M., Guisez, Y., Caubergs, R., & Horemans, N. (2006). Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. *Phytochemistry*, 67(12), 1185-1195.
- Hoskin, S. O., & Gee, E. K. (2004). Feeding value of pastures for horses. *New Zealand Veterinary Journal*, 52(6), 332-341. doi: 10.1080/00480169.2004.36449
- Haupt, K., Haupt, T. R., Johnson, J. L., Erb, H. N., & Yeon, S. C. (2001). The Effect of Exercise Deprivation on the Behaviour and Physiology of Straight Stall Confined Pregnant Mares. *Animal Welfare*, 10(3), 257-267.
- Jackson, S. A., Rich, V. A., Ralston, S. L., & Anderson, E. W. (1984). Feeding behavior and feed efficiency in groups of horses as a function of feeding frequency and the use of alfalfa cubes. *J. Anim. Sci*, 59(Suppl 1), 152.
- Janis, C. (1976). The Evolutionary Strategy of the Equidae and the Origins of Rumen and Cecal Digestion. *Evolution*, 30(4), 757-774.
- Jonsson, A. (1989). The role of yeasts and Clostridia in silage deteriorations. *Rapport nr. 42. Sveriges Lantbruksuniversitet*, 11–22. .
- Jørgensen, M., Schjelderup, I., & Junttila, O. (1994). Dry matter production and botanical composition of monocultures and mixtures of meadow fescue (*Festuca pratensis* Huds.) and timothy (*Phleum pratense* L.) in field experiments at three locations in northern Norway 1984-89. *Norwegian Journal of Agricultural Sciences*, 8.

- Kern, D., & Bond, J. (1972). Eating patterns of ponies fed diets ad libitum. *Journal of Animal Science Abstract*, 35, 285.
- Kunelius, H. T., Dürr, G. H., McRae, K. B., & Fillmore, S. A. E. (2006). Performance of Timothy-based Grass/Legume Mixtures in Cold Winter Region. *Journal of Agronomy and Crop Science*, 192(3), 159-167. doi: 10.1111/j.1439-037X.2006.00195.x
- Kunelius, H. T., Durr, G. H., McRae, K. B., Fillmore, S. A. E., Belanger, G., & Papadopoulos, Y. A. (2003). Yield, herbage composition, and tillering of timothy cultivars under grazing. [Article]. *Canadian Journal of Plant Science*, 83(1), 57-63.
- Kusunose, R. (1992). Diurnal pattern of cribbing in stabled horses. *Japanese Journal of Equine Science*, 3(2), 3.
- LaCasha, P. A., Brady, H. A., Allen, V. G., Richardson, C. R., & Pond, K. R. (1999). Voluntary intake, digestibility, and subsequent selection of Matua bromegrass, coastal bermudagrass, and alfalfa hays by yearling horses. *Journal of Animal Science*, 77(10), 2766-2773.
- Langer, R. H. M. (1990). *Pastures: their ecology and management*: Oxford University Press Auckland^ eNew Zealand New Zealand.
- Lathrop, A., & Bohstedt, G. (1938). Oat mill feed.
- Lawrence, L., JASTER, E., WISCHOVER, L., MOORE, K., & HINTZ, H. (1987). Acceptability of alfalfa hay treated with an organic acid preservative for horses. *Canadian Journal of Animal Science*, 67(1), 217-220.
- Lewis, L. D. (1995). *Equine Clinical Nutrition : Feeding and Care*. PA, USA: Williams & Wilkins.
- Lindberg, A. C., Kelland, A., & Nicol, C. J. (1999). Effects of observational learning on acquisition of an operant response in horses. *Applied Animal Behaviour Science*, 61(3), 187-199. doi: 10.1016/s0168-1591(98)00184-1
- Lindberg, J. (1988). Influence of cutting time and N-fertilization on the nutritive value of timothy, 2: Estimates of rumen degradability of nitrogenous compounds. *Swedish Journal of Agricultural Research*, 18.
- Lindsey, J., Beals, C., & Archibald, J. (1926). The digestibility and energy values of feeds for horses. *J. Agr. Res*, 32, 569.
- Luescher, U. A., McKeown, D. B., & Dean, H. (1998). A cross-sectional study on compulsive behaviour (stable vices) in horses. *Equine Veterinary Journal*, 30(S27), 14-18. doi: 10.1111/j.2042-3306.1998.tb05138.x
- McAfee, L. M., Mills, D. S., & Cooper, J. J. (2002). The use of mirrors for the control of stereotypic weaving behaviour in the stabled horse. *Applied Animal Behaviour Science*, 78(2-4), 159-173. doi: 10.1016/s0168-1591(02)00086-2
- Mccall, C. (2009). Equine Behavior: A Nutritional Link? *Advances in equine nutrition.*, Auburn University, Alabama.
- McCann, J., & Hoveland, C. (1991). Equine grazing preferences among winter annual grasses and clovers adapted to the southeastern United States. *Journal of Equine Veterinary Science*, 11(5), 275-277.
- McClure, S. R., Chaffin, M. K., & Beaver, B. V. (1992). Nonpharmacologic management of stereotypic self-mutilative behavior in a stallion. *Journal of the American Veterinary Medical Association*, 200(12), 1975.
- McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., & Morgan, C. A. (2002). *Animal nutrition* (6th ed.). Harlow: UK Prentice Hall.

- McDonnell, S. M., & Diehl, N. K. (1990). Computer-assisted recording of live and videotaped horse behavior: reliability studies. *Applied Animal Behaviour Science*, 27(1), 1-7.
- McDonnell, S. M., Freeman, D. A., Cymbaluk, N. F., Schott, H. C. I., Hinchcliff, K., & Kyle, B. (1999). Behavior of stabled horses provided continuous or intermittent access to drinking water. *American Journal of veterinary research*, 60(11), 1451-1456.
- McDonnell, S. M., & Haviland, J. C. S. (1995). Agonistic ethogram of the equid bachelor band. *Applied Animal Behaviour Science*, 43(3), 147-188.
- McGreevy, P. (2004). *Equine Behaviour*. Philadelphia, USA: Elsevier.
- McGreevy, P., Cripps, P., French, N., Green, L., & Nicol, C. J. (2010). Management factors associated with stereotypic and redirected behaviour in the Thoroughbred horse. *Equine Veterinary Journal*, 27(2), 86-91.
- McGreevy, P., Cripps, P. J., French, N. P., Green, L. E., & Nicol, C. J. (2010). Management factors associated with stereotypic and redirected behaviour in the Thoroughbred horse. *Equine Veterinary Journal*, 27(2), 86-91.
- Medina, B., Drogoul, C., & Julliand, V. (2002). *Feeding practices and digestive transit*. Paper presented at the 28ème journée de la recherche équine 27 février 2002.
- Meyer, H., Ahlswede, L., & Reinhardt, H. J. (1975). Time of feed uptake, masticatory frequency, and mincing of feed in horses. *UNTERSUCHUNGEN UBER FRESSDAUER, KAUFREQUENZ UND FUTTERZERKLEINERUNG BEIM PFERD*, 82(2), 54-58.
- Miller, D. F. (1958). *Composition of cereal grains and forages* (Vol. 585): National Academies.
- Moehlman, P. D. R. (1974). *Behavior and ecology of feral asses (Equus asinus)*: University of Wisconsin--Madison.
- Moore-Colyer, M. J. S., & Longland, A. C. (2000). Intakes and in vivo apparent digestibilities of four types of conserved grass forage by ponies. *Journal of Animal Science*, 71, 527-534. doi: 1357-7298/00/07890527
- Moore-Colyer, M. J. S., Longland, A. C., & Morrow, H. J. (2003). Mathematical modelling of digesta passage rate, mean retention time and in vivo apparent digestibility of two different lengths of hay and big-bale grass silage in ponies. *British Journal of Nutrition*, 90, 109-118. doi: 10.1079/BJN2003869
- Mroz, Z., Bakker, G. C., Jongbloed, A. W., Dekker, R. A., Jongbloed, R., & van Beers, A. (1996). Apparent digestibility of nutrients in diets with different energy density, as estimated by direct and marker methods for pigs with or without ileo-cecal cannulas. *Journal of Animal Science*, 74(2), 403-412.
- Muhonen, S., Lindberg, J., Bertilsson, J., & Jansson, A. (2009). Effects on fluid balance, digestion and exercise response in Standardbred horses fed silage, haylage and hay. *energy*, 5, 7.
- Muir, H. E., Murray, S. M., Fahey, G. C., Merchen, N. R., & Reinhart, G. A. (1996). Nutrient digestion by ileal cannulated dogs as affected by dietary fibers with various fermentation characteristics. *Journal of Animal Science*, 74(7), 1641-1648.
- Müller, C. E. (2005). Fermentation patterns of small-bale silage and haylage produced as a feed for horses. *Grass and Forage Science*, 60(2), 109-118. doi: 10.1111/j.1365-2494.2005.00457.x

- Müller, C. E., Möller, J., Jensen, S. K., & Udén, P. (2007). Tocopherol and carotenoid levels in baled silage and haylage in relation to horse requirements. *Animal Feed Science and Technology*, 137(1–2), 182-197. doi: 10.1016/j.anifeedsci.2006.10.007
- Müller, C. E., & Udén, P. (2007). Preference of horses for grass conserved as hay, haylage or silage. *Animal Feed Science and Technology*, 132(1–2), 66-78. doi: 10.1016/j.anifeedsci.2006.02.013
- Nicholson, J., & Friend, D. (1965). The digestibility of potato pulp protein by some species of farm animals. *Canadian Journal of Animal Science*, 45(3), 141-145.
- Nicol, C. (1999). Understanding equine stereotypies. *Equine Veterinary Journal*, 31(S28), 20-25.
- Ninomiya, S., Kusunose, R., Sato, S., Terada, M., & Sugawara, K. (2004). Effects of feeding methods on eating frustration in stabled horses. *Animal Science Journal*, 75(5), 465-469. doi: 10.1111/j.1740-0929.2004.00214.x
- Ninomiya, S., Sato, S., Kusunose, R., Mitumasu, T., & Obara, Y. (2007). A note on a behavioural indicator of satisfaction in stabled horses. *Applied Animal Behaviour Science*, 106(1–3), 184-189. doi: 10.1016/j.applanim.2006.06.011
- Ninomiya, S., Sato, S., & Sugawara, K. (2007). Weaving in stabled horses and its relationship to other behavioural traits. *Applied Animal Behaviour Science*, 106(1–3), 134-143. doi: 10.1016/j.applanim.2006.06.014
- Notermans, S., Kozaki, S., & Vanschothorst, M. (1979). TOXIN PRODUCTION BY CLOSTRIDIUM-BOTULINUM IN GRASS. [Article]. *Applied and Environmental Microbiology*, 38(5), 767-771.
- NRC. (1989) *Nutrient Requirements of Horses* (6th ed.). Washington DC . The National Academy Press.
- NRC. (2007) *Nutrient Requirement of Horses* (6th ed.). Washington, DC: The National Academies Press.
- Ödberg, F. O., & Francis-Smith, K. (1977). Studies on the formation of ungrazed eliminative areas in fields used by horses. *Applied Animal Ethology*, 3(1), 27-34. doi: 10.1016/0304-3762(77)90068-2
- Olsson, N., Ruudvere, A., Abstracts, N., & Reviews, V. (1955). *The Nutrition of the Horse*, By N. Olsson and A. Ruudvere.
- Ordakowski-Burk, A. L., Quinn, R. W., Shellem, T. A., & Vough, L. R. (2006). Voluntary intake and digestibility of reed canarygrass and timothy hay fed to horses. *Journal of Animal Science*, 84(11), 3104-3109. doi: 10.2527/jas.2005-607
- Ordakowski, A. L., Kronfeld, D. S., Holland, J. L., Hargreaves, B. J., Gay, L. S., Harris, P. A., . . . Sklan, D. (2001). Alkanes as internal markers to estimate digestibility of hay or hay plus concentrate diets in horses. *Journal of Animal Science*, 79(6), 1516-1522.
- Pagan, J. D., Harris, P., Brewster-Barnes, T., Duren, S. E., & Jackson, S. G. (1998). Exercise Affects Digestibility and Rate of Passage of All-Forage and Mixed Diets in Thoroughbred Horses. *The Journal of Nutrition*, 128(12), 2704S-2707S.
- Parker, R. (2008). *Equine Science* (3rd ed.). New York: Delmar publication.
- Ragnarsson, S., & Lindberg, J. E. (2008). Nutritional value of timothy haylage in Icelandic horses. 113(2), 202-208.
- Ragnarsson, S., & Lindberg, J. E. (2010a). Nutritional value of mixed grass haylage in Icelandic horses. *Livestock Science*, 131(1), 83-87.

- Ragnarsson, S., & Lindberg, J. E. (2010b). ORIGINAL ARTICLE: Impact of feeding level on digestibility of a haylage-only diet in Icelandic horses. *Journal of Animal Physiology and Animal Nutrition*, 94(5), 623-627. doi: 10.1111/j.1439-0396.2009.00947.x
- Ralston, S. L. (1984). Controls of Feeding in Horses. *Journal of Animal Science*, 59(5), 1354-1361.
- Ralston, S. L. (1986). Feeding behavior. *The Veterinary clinics of North America. Equine practice*, 2(3), 609-621.
- Ralston, S. L., & Baile, C. A. (1983). Factors in the control of feed intake of horses and ponies. *Neuroscience & Biobehavioral Reviews*, 7(4), 465-470. doi: 10.1016/0149-7634(83)90024-6
- Ralston, S. L., & McKenzie, S. (1992). Effect of carbohydrate intake on behavioral reactivity and activity levels in horses. *J. Anim. Sci*, 70(Suppl 1), 159.
- Ralston, S. L., Van den Broek, G., & Baile, C. A. (1979). Feed Intake Patterns and Associated Blood Glucose, Free Fatty Acid and Insulin Changes in Ponies. *Journal of Animal Science*, 49(3), 838-845.
- Ricketts, S. W., Greet, T. R. C., Glyn, P. J., Ginnett, C. D. R., McAllister, E. P., McCaig, J., . . . Murray, L. G. (1984). 13 CASES OF BOTULISM IN HORSES FED BIG BALE SILAGE. [Article]. *Equine Veterinary Journal*, 16(6), 515-518.
- Rogalski, M. (1984). Effect of carbohydrates and lignin on preferences for and intakes of pasture plants by mares. *Roczniki Akademii Rolniczej w Poznaniu*, 27, 183-193.
- Ruckerbusch, Y. (1975). The hypnogram as an index of adaptation of farm animals to changes in their environment. *Applied Animal Ethology*, 2(1), 3-18. doi: 10.1016/0304-3762(75)90061-9
- Salter, R. E., & Pluth, D. J. (1980). Determinants of mineral lick utilization by Feral Horses. *Northwest Science*, 54, 109-118.
- Seshan, P., & Sen, K. (1942). Studies on Carotene in relation to animal nutrition: Part III. Stability of carotene in plant material with special reference to hay making and storage. *The Journal of Agricultural Science*, 32(03), 275-285.
- Shewmaker, G. E., Undersander, D., Lawrence, L. M., & Lacefield, G. D. (2005). Alfalfa: The High Quality Hay for Horses. Retrieved 15/03/2012, 2012, from [http://www.alfalfa.org/pdf/Alfalfa%20fo%20Horses%20\(low%20res\).pdf](http://www.alfalfa.org/pdf/Alfalfa%20fo%20Horses%20(low%20res).pdf)
- Small, E. (1996). Adaptations to herbivory in alfalfa (*Medicago sativa*). *Canadian Journal of Botany*, 74(6), 807-822.
- Staniar, W. B., Bussard, J. R., Repard, N. M., Hall, M. H., & Burk, A. O. (2010). Voluntary intake and digestibility of teff hay fed to horses. *Journal of Animal Science*, 88(10), 3296-3303. doi: 10.2527/jas.2009-2668
- Sutton, E. I., Bowland, J. P., & McCarthy, J. F. (1977). STUDIES WITH HORSES COMPARING 4N-HCl INSOLUBLE ASH AS AN INDEX MATERIAL WITH TOTAL FECAL COLLECTION IN THE DETERMINATION OF APPARENT DIGESTIBILITIES. *Canadian Journal of Animal Science*, 57(3), 543-549. doi: 10.4141/cjas77-070
- Sweeting, M. P., Houpt, C. E., & Houpt, K. A. (1985). Social Facilitation of Feeding and Time Budgets in Stabled Ponies. *Journal of Animal Science*, 60(2), 369-374.
- Thorne, J. B., Goodwin, D., Kennedy, M. J., Davidson, H. P. B., & Harris, P. (2005). Foraging enrichment for individually housed horses: Practicality and effects on

- behaviour. *Applied Animal Behaviour Science*, 94(1-2), 149-164. doi: 10.1016/j.applanim.2005.02.002
- Tyler, S. J. (1972). The behavior and social organization of the New Forest ponies. *Animal Behaviour Monographs*, 5 (2), 85-344.
- Van Soest, P. (1965). Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. *Journal of Animal Science*, 24(3), 834-843.
- Van Soest, P. J. (1994). *Nutritional ecology of the ruminant*: Cornell University Press.
- Vandenput, S., Istasse, L., Nicks, B., & Lekeux, P. (1997). Airborne dust and aeroallergen concentrations in different sources of feed and bedding for horses. *Veterinary quarterly*, 19(4), 154-158.
- Waghorn, G., & Clark, D. (2004). Feeding value of pastures for ruminants. *New Zealand Veterinary Journal*, 52(6), 320-331.
- Waring, G. H. (2003). *Horse Behaviour* (2nd ed.). Norwich, New York, USA: William Andrew Publishing.
- Waters, A. J., Nicol, C. J., & French, N. P. (2002). Factors influencing the development of stereotypic and redirected behaviours in young horses: Findings of a four year prospective epidemiological study. *Equine Veterinary Journal*, 34(6), 572-579.
- Wilkins, R. (1974). *The nutritive value of silages*. Paper presented at the Proceedings of the Eighth Nutrition Conference for Feed Manufacturers, Butterworths, Nottingham.
- Wilkinson, J. M. (2005). *Silage*. Lincoln, United Kingdom: Chalcombe.
- Willard, J. G., Willard, J., Wolfram, S., & Baker, J. (1977). Effect of diet on caecal pH and Feeding behaviour of horses. *Journal of Animal Science*, 45(1).
- Wolff, A., Hausberger, M., & Le Scolan, N. (1997). Experimental tests to assess emotionality in horses. *Behavioural Processes*, 40(3), 209-221. doi: [http://dx.doi.org/10.1016/S0376-6357\(97\)00784-5](http://dx.doi.org/10.1016/S0376-6357(97)00784-5)

## CHAPTER 5 - General Discussion

This thesis describes a field study and laboratory study and two animal studies evaluating the suitability of timothy grass as a viable crop grown under New Zealand conditions, the composition of an ensiled lucerne and timothy mix an ensiled timothy product, the stability of an ensiled lucerne and timothy mix product during 12 month storage and also the suitability of an ensiled lucerne and timothy mix, ensiled timothy and Rye Clover hay feed for consumption by Thoroughbred horses.

The initial series of studies were carried out to monitor how timothy grass grows under New Zealand conditions, to analyse the nutrient content of an ensiled timothy product and an ensiled product combining timothy with lucerne (FiberEzy®) and to assess the storage characteristics of FiberEzy®.

Grass samples were collected from a representative section (6m x 16m) of field in Reporoa, (Waikato, New Zealand) planted with timothy grass (*Phleum pratense L.*) on the 10th of March 2010 at Longitude 176°34'E, Latitude 38°39'S. The samples were collected at regular intervals to investigate changes in the dry matter, acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin content of the crop. Once harvested and ensiled, a bag of timothy and FiberEzy® were analysed for total dry matter, percentage crude protein, percentage crude fat, percentage crude fibre, percentage ash, gross energy percentage hot water soluble carbohydrates, percentage pectin, percentage neutral detergent fibre (NDF), percentage acid detergent fibre (ADF), percentage lignin and vitamin E content. A shelf life stability study of FiberEzy® was carried out by sub-sampling a bag of the product every 3 months and analysing for total dry matter, percentage crude protein, percentage hot water soluble carbohydrates and vitamin E (mg/kg) over 12 months of storage.

There was an increase (17.7-35.9%) in the dry matter content and lignin content (3.0-6.3%) of timothy over the various harvest periods. Lab analysis of FiberEzy® versus timothy showed that FiberEzy® had higher levels of crude protein, pectin, lignin, ash and vitamin E ( $p < 0.05$ ), and lower levels of crude fibre and neutral detergent fibre

( $P < 0.05$ ) than the timothy grass. During the storage trial, analysis of FiberEzy® showed an increase in the content of vitamin E (37.6- 124.9mg/kg) over time.

The addition of lucerne to timothy grass in the ensiled FiberEzy® product resulted in higher crude protein levels and lower levels of fibre as expected. Shelf storage of FiberEzy® resulted in a significant increase in the Vitamin E content over time due to microbial action in the ensiled forages.

The first animal study in the thesis was carried out to determine the apparent digestibility of dry matter (DM) and gross energy (GE) in ensiled FiberEzy® (lucerne and timothy) and timothy (*Phleum pratense*) haylage fed to four Thoroughbred horses.

FiberEzy® and timothy haylage was offered at 2.5% of the body weight as DM to four Thoroughbred horses ( $10 \pm 1$  yr.;  $562.5 \pm 30.7$  kg initial BW). The horses were stalled individually in loose boxes (4 x 4 m) lined with rubber matting. Horses were randomly paired and assigned to be fed FiberEzy® (FE) or timothy (T) for two 18 day periods, in a 2 x 2 Latin square experimental design. Each 18 day period comprised of a 14 day dietary adaptation period where the horses were habituated to the pens and diet and a 4 d (96 h) total faecal collection. The apparent digestibility of DM and GE were measured on days 15- 18; at 12 hourly intervals faecal material from each horse was collected, weighed, and a subsample was taken and frozen at  $-20^{\circ}\text{C}$ . One kg samples of the feed were collected and frozen at  $-20^{\circ}\text{C}$  on day 15.

The energy content of the FiberEzy® and timothy haylage varied between the first and second part of the study, with the DE of timothy varying from 6.9 MJ/kg DM to 9.4 MJ/kg DM. Total energy intake of FiberEzy® and timothy haylage varied between horses, and between groups, with horses fed timothy after FiberEzy® reducing their total energy intake. However, the apparent digestibility of FiberEzy® tended to be greater than for timothy.

The final animal study aimed to determine the voluntary feed intake and behavioural observation of six Thoroughbred horses being fed FiberEzy® and Rye clover hay.

Six Thoroughbred geldings, ( $10 \pm 1$  yr.;  $550.6 \pm 15.8$  kg) were offered FiberEzy® (FE) or Rye-clover hay (RCH) at >3% of the body weight. The horses were stalled individually in loose boxes (4 x 4 m) lined with rubber matting. Voluntary feed intake was measured over 17 days from days 9-17 of the study during which the horses were scan sampled and videoed for two hours every morning (9:30- 11:30 am) after feeding and two hours in the evening (4:30-6:30 pm) after feeding.

There were significant differences between time periods ( $24.3 \pm 0.9$  vs  $17.8 \pm 0.4$  kg DM /day,  $p < 0.05$ ) and feeds for VFI (FE  $24.3 \pm 0.4$  vs. RCH  $17.8 \pm 0.7$  kg DM /day) but not an interaction. Behaviours were typical of loose box housed horses and differences in feeding behaviour observed correlated with the differences in VFI measured between feeds. The greater VFI on the FiberEzy® diet and this was associated with a greater eating behaviour observed in the horses. The ensiled product FiberEzy® appears a suitable feed based on VFI and behaviour for feeding to horses.