EFFECTS OF PSYLLIUM SUPPLEMENTATION ON SERUM PROTEIN,

TRIGLYCERIDES, ELECTROLYTES AND PACKED CELL VOLUME

IN HORSES GRAZING RAPIDLY GROWING COOL

SEASON GRASSES

by

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ABSTRACT

The effect of psyllium supplementation on absorption of nutrients other than glucose and water in the intestine of horses is not known. Eleven 10- to 18-yr-old light breed stock horses were used in a completely randomized design to determine the effects of psyllium supplementation on serum protein, serum triglyceride and serum electrolyte concentrations as well as PCV, water intake and feed intake. Horses were grazed individually separated with electric tape for 8 h every day. At night they were kept in individual dry lots where they received a mixed grain and a psyllium supplement. Psyllium treatment level was 1) 180 g/d psyllium or 2) an isocaloric control and 0g/d psyllium. Blood samples were collected on day 8, 15, 22, and 29 of the study and used to determine protein and triglyceride concentrations. Concentrations of serum electrolytes and packed cell volumes (PCV) were measured using blood collected on day 0, 8, 15, 22, and 29. Horses that received psyllium showed reduced concentrations of triglycerides ($P < 0.001$) and protein ($P = 0.019$) compared to those receiving an isocaloric control. No differences ($P > 0.065$) in the lysine and arginine concentrations were found between treatments. There were no differences ($P > 0.085$) in chloride and sodium concentrations, PCVs, and water and feed intake between treatments. There was a treatment by day interaction ($P = 0.032$) for concentrations of potassium; furthermore, the treatment effect on potassium concentrations existed only on study days 8, and 22, while no difference was found on collection days 0, 15, and 29. Supplementing horses with 180 g/d psyllium appears to have a lowering effect on serum protein and triglyceride concentrations in the intestine of horses.

CHAPTER 1

INTRODUCTION

Psyllium is a commercially marketed product that has a history of being used as a supplemental dietary fiber in humans and a treatment to remove large colon sand from horses (Dhar et al., 2005; Hotwagner and Iben, 2008). Sand impaction has long been known to cause colic in horses in various geographic areas of the United States (Kaneene et al., 1997; Udenberg, 1979). The gel-like substance formed by psyllium is thought to help horses pass ingested sand and dirt into the large colon.

Psyllium supplementation in humans diagnosed with diabetes type 2, appeared to lower glucose and total lipid concentrations (Anderson et al., 1999; Rodríguez-Morán et al., 1998). Recent research by Moreaux et al. in 2011, indicated similar effects in horses; psyllium lowered blood glucose concentrations in horses. They concluded that psyllium could be a tool to manage horses with equine metabolic syndrome and prevent development of laminitis. Laminitis is a painful condition in equiids that is characterized by hoof wall detachment from underlying tissue. It can become so severe that the hoof will not support the animal's weight (Sillence et al., 2007). It has been recommended that the most effective method to prevent laminitis is to reduce the blood glucose concentration or increase insulin sensitivity (Rural Industries Research and Development Corporation, 2010).

It has been documented that psyllium supplementation decreased triglyceride concentrations in humans (Rodríguez-Morán et al., 1998) and glucose concentrations in

humans and horses (Rodríguez-Morán et al., 1998; Moreaux et al., 2011); however, the physiological mechanisms for these effects are not known. Furthermore, the effects of psyllium on these variables may be related to its influence of intestinal absorption of other nutrients. If psyllium reduces absorption of these nutrients, nutrient deficiencies might result. Therefore, the objective of this study was to identify the effects of psyllium supplementation on total feed intake and serum protein and triglycerides concentrations in the horse.

Additionally, in a previous study, it was reported that psyllium supplementation might improve the hydration state of horses (Cinotti et al., 1997). They suggested that psyllium increased water availability in the gut and together with the slower passage rate induced by psyllium allowed the intestine to absorb more fluid. Thus, another objective of this study was to evaluate the effects of psyllium supplementation on the hydration state of the horses.

CHAPTER 2

LITERATURE REVIEW

<u>Psyllium</u>

Plantago ovata is a short-stemmed annual herb that is cultivated primarily in India (Dhar et al., 2005). The seed husk of *Plantago ovata*, a thin white membrane covering the concave side of the seed, is commonly called "psyllium" (Dhar et al., 2005). Psyllium is a commercially marketed product that has a history of being used as a supplemental dietary fiber in humans and a treatment to remove large colon sand from horses (Dhar et al., 2005; Hotwagner and Iben, 2008). Dietary fiber is commonly defined as plant material that is water-soluble and resists digestion by the enzymes of the human gastrointestinal tract (Blackwood et al., 2000).

Psyllium contains a gel forming fraction, consisting of non-nutrient polysaccharides, that resists digestion by the intestinal microflora of humans and other animals (Fischer et al., 2004). With mild alkali treatment, the seed husk yields 85% polysaccharide (single species) and 15% non-polysaccharides (Sandhu and Hudson, 1981). The polysaccharide fraction forms a gel in water, and is classified as mucilage.

The composition of the mucilage formed by psyllium was first studied as early as 1935, with the conclusion that it consists of a mixture of polyuronides (Anderson and Fireman, 1935). More recently it has been determined that the mucilage contained in psyllium is an arabinosyl (galactosyluronic acid) rhamnosyxylan (Sandhu and Hudson, 1981). The mucilage of psyllium can absorb many times its own weight of water and is

closely related to the health benefits and applications of psyllium (Anderson and

Fireman, 1935; Sandhu and Hudson, 1981; Guo, 2009).

<div align="center">Psyllium as a Treatment for and Preventative of Sand Colic</div>

Consumption of sand and dirt can lead to diarrhea, weight loss, and colic in horses

(Hotwagner and Iben, 2008). It is a common practice to supplement horses that are

predisposed to consume sand with psyllium to treat and prevent these effects. The gel-

like substance formed by psyllium is thought to assist the horse to pass ingested sand and

dirt through the large colon. Recently, psyllium has been found to significantly increase

fecal sand output of horses ingesting sand (Landes et al., 2008; Hotwagner and Iben,

2008). Therefore, psyllium is thought to have the ability to treat and prevent sand colic in

horses.

However, the available evidence regarding the ability of psyllium to treat and

prevent sand colic are controversial. Hammock et al. (1998) found that psyllium had no

effect on removing sand placed surgically into the large intestine of ponies. These results

suggest that psyllium does not have the ability to remove sand that has already

accumulated in the large intestine. There is one report that indicates that the response to

psyllium varies greatly among individuals (Ruohoniemi et al., 2001). In some horses, the

response to psyllium treatment was quick and complete, while in others it only reduced

the amount of sand and did not seem to be effective if the surface of the accumulation

became distinct (Ruohoniemi et al., 2001). Additionally, there is some concern that the

effectiveness of psyllium supplementation is reduced when used as a long-term treatment,

since the colonic flora might adapt to it and improve its ability to degrade psyllium (Campbell and Fahey, 1997; Hammock et al., 1998).

Application of Psyllium as a Dietary Supplement

Due to its gel forming and water binding properties, psyllium has been used in humans to treat constipation as well as diarrhea, inflammatory bowel disease-ulcerative colitis (Crohn's disease), and irritable bowel syndrome (Singh, 2007; Attaluri et al., 2011; Washington et al, 1998). The intake of psyllium is thought to increase the viscosity of intestinal contents (Dikeman et al., 2006), which decreases the rate of absorption of glucose and other nutrients in humans (Blackwood et al., 2000). Research supports the use of psyllium in human patients with diabetes. In their study in 1999, Anderson et al. determined that glucose and lipid concentrations in men with diabetes type 2 were decreased significantly when 5.1g of psyllium were consumed twice daily. Similar effects of psyllium supplementation on diabetes type 2 patients were reported by Rodríguez-Morán et al. (1998).

While the prevalence of obesity, insulin resistance, and diabetes type 2 in human populations in developed countries is rising rapidly; the horse industry is facing similar problems. Both obesity and insulin resistance are correlated with the development of laminitis in horses (Frank, 2006). A similar condition to diabetes in humans exists in horses, where horses have difficulties processing glucose. It is known as equine metabolic syndrome. Psyllium has been shown to reduce blood glucose and insulin concentrations in horses on a twice daily feeding schedule (Moreaux et al., 2011).

Therefore, psyllium might be a nutritional tool to prevent equine metabolic disease and laminitis in horses. However, the effect of psyllium supplementation on nutrients other than glucose in the horse has not been studied. Furthermore, use of psyllium may adversely impact essential nutrient absorption and lead to nutritional deficits.

<u>Digestion in the Horse</u>

Horses are non-ruminant herbivores that digest forage fiber in the hindgut. The hindgut can be divided into 6 segments: caecum, right ventral colon, left ventral colon, left dorsal colon, right dorsal colon, and small colon. The foregut includes the stomach and the small intestines (duodenum, jejunum, ileum), and is mainly responsible for processing and transport of soluble or easily digestible nutrients such as carbohydrates, fatty acids and AA (for review see, Ellis, 2005).

The equine stomach normally has a capacity of 8 to 16 L. While fluids leave the stomach quickly, feed particles remain there for over 48 h while digestion is initiated by hydrochloric acid and contractions of the stomach muscles (Moore, 2001). The stomach is also colonized by various microorganisms, mainly those that show the ability to ferment starch and highly fermentable carbohydrates (de Fombelle et al., 2003; Varloud et al., 2007).

In a study by Varloud et al. in 2004, a mean length of 1,456 cm was measured for the small intestine, which made up for approximately 70% of the total gut length. Moore et al. (2001) determined an approximate length of 24 m for the small intestine. The main function of the horse's small intestine is the digestion and absorption of soluble

carbohydrates, while the hindgut is mainly responsible for digestion of fiber. While the passage rate of digesta through the stomach is rapid in horses, a transit time of 3.9 to 4.7 h in the small intestine and 20.9 to 37.5 h in the large intestine has been reported (Medina et al., 2002).

Digestion of Fiber

Plant structural carbohydrates such as cellulose, hemicellulose, and other β-linked polymers are resistant to pre-cecal digestion in horses but instead are digested by anaerobic microbial fermentation (Hintz et al., 1971). In horses, the hindgut is the main site of microbial fermentation and absorption of fermentation products. The ability to digest plant cell-walls is low in the foregut and high in the hindgut of horses (Varloud et al., 2004). Fermentation in the hind gut produces volatile fatty acids (VFA), which are readily absorbed and are a main energy source for the horse (see review, Hintz, 1994). The horse digests fiber less efficiently than a cow, which could be due to the faster passage rate of horses or because ruminal microbes ferment most forages at a faster rate (Koller et al., 1978).

When high grain diets are fed, microbial VFA production decreases and relatively more carbohydrate is digested in the small intestine and absorbed as glucose (Hintz et al., 1971). While the stomach and small intestine of horses mainly hosts lactobacilli, streptococci and lactate-utilizing bacteria, which are correlated with the digestion of readily fermentable carbohydrates, the hindgut has been found to have the highest concentration of cellulolytic bacteria compared to other segments of the digestive tract (de Fombelle et al., 2003). Fermentation by cellulolytic bacteria in the hindgut produces

VFA's. Subsequently, greater VFA concentrations have been observed in the large intestine compared to other sites (de Fombelle et al., 2003).

There appears to be considerable variation in the apparent digestibility of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in different segments of the large intestine of the horse (de Fombelle et al., 2003; Varloud et al., 2004). However, this is equivocal. One study reported that fiber digestibility for both NDF and ADF seems to increase along the hindgut with the highest digestibility in the left dorsal colon (Varloud et al. 2004). Another study found the highest fiber degradation in the ceacum, but each anatomical segment of the colon was also involved, especially when large amounts of fiber were ingested (de Fombelle et al., 2003).

Protein

"Proteins are polymers composed of hundreds or even thousands of amino acids linked in series by peptide bonds" (Garrett and Grisham, 2010). Proteins differentiate depending on the type of AA incorporated into a chain as well as the length of the chain, therefore the horse's requirement is actually for AA rather than protein as a whole (NRC, 2007). Mammals lack the ability to synthesize all AA that are required for protein synthesis. Therefore, they depend on the intake of certain AA from the diet (Ellis and Hill, 2005). The following 10 AA are thought to be essential for horses: arginine, lysine, threonine, leucine, isoleucine, valine, methionine, phenylalanine, tryptophan and histidine (NRC, 2007). The requirements for crude protein for maintenance are estimated at 630 g/d for a 500 kg horse. Crude protein needed above maintenance is estimated at

approximately 232 g/d for 500 kg horse receiving heavy exercise and 375 g/d for a horse in very heavy exercise (NRC, 2007).

Lysine, which has been determined to be the first limiting AA in horses, should always make up 4.3% of crude protein intake per day. This implies that requirements for maintenance are estimated at a minimum of 18 g and optimum of 27 g for a 500 kg horse. Requirements for horses receiving exercise and for growing horses are consequently higher (NRC, 2007). In a study by Graham et al. in 1994, those yearling horses that received supplemental lysine showed significant increases in daily muscle gain and protein synthesis compared to horses receiving a basal diet without added lysine. Also, they reported that the growth would increase even more if in addition to the lysine, threonine was supplemented. They concluded that threonine could be the second limiting AA for yearling horses.

The protein requirements of horses for physical activity above maintenance have been controversial for some time. The NRC in 1978 indicated that protein requirements for exercising and non-exercising horses do not differ, whereas the NRC in 2007 accounts for the effects of regular exercise on the accretion of lean body mass and for the loss of protein in sweat (NRC, 1978; NRC, 2007). It is a common practice to feed additional grain concentrate to performance horses, which provides extra protein for those horses (Patterson et al., 1985).

The digestion of dietary protein starts in the stomach, where the pro-enzyme pepsinogen is secreted and converted to pepsin A by autocatalysis. Pepsin A is used in the first stage of proteolysis (Ellis and Hill, 2005). However, the main site of proteolysis

in horses is the duodenum. Endo- and exopeptidase are released in the intestine from the pancreas and their activity leads to the release of AA, di-peptides and tri-peptides (Ellis and Hill, 2005).

There are two different sites of protein digestion in the horse. The protein digested and absorbed from the small intestine contributes to the AA pool. Some dietary protein is not absorbed in the small intestine and reaches the hindgut; this protein is degraded to ammonia by microbial fermentation (Hintz, 1994). Opinions on whether AA from microbial protein synthesis can be absorbed in the large intestine of the horse vary; but previous research indicates that absorption is unlikely (McMeniman et al., 1987; Bochröder et al., 1994). Bochröder et al. (1994) studied the transport of lysine, arginine and histidine across the mucosa of the equine colon *in vitro*. Based on their findings they excluded active transport mechanisms as well as any significant diffusion process. Cecal infusions of protein sources did not improve the nitrogen balance in horses in the study of Reitnour and Salsbury (1972), which leads to the idea that the hindgut is not of significant importance in protein or AA absorption.

Triglycerides

Triglycerides (also called triacylglycerols) are the most common storage form and energy reserve of fat in animals. They are stored in specialized cells called adipocytes or adipose cells (Garrett and Grisham, 2010). "Most natural plant and animal fat is composed of mixtures of simple and mixed triacylglycerols", where simple triglycerides (TAG) are molecules that consist of glycerol esterified with three fatty acids that are all

the same, while mixed TAG contain two or three different fatty acids (Garrett and Grisham, 2010).

In horses, dietary fat essentially reaches the small intestine in the form of triglycerides. Pancreatic lipases in the small intestine cleave the lipid into its individual monoglycerides which are absorbed efficiently (Meyer, 1995). Once absorbed, TAG is resynthesised and together with protein and phospholipids form chylomicrons, which transport dietary lipids from the intestine to 3 main locations in the body (Geelen et al. 1999). These are, in order of amount of triglycerides typically stored, adipose tissue, skeletal muscle, and liver (Frayn et al., 2006). Due to the action of lipoprotein lipase, the esterified fatty acids of chylomicrons are stored in the adipose tissue of the body (Geelen et al., 1999). From there they can be released again during energy shortage to be utilized by the liver, which esterifies them back into TAG, which then can be used by skeleton and heart muscle for oxidation (Frayn et al., 2006).

Since the diet of the horse is generally poor in lipid, supplementation of lipid from external sources is common in equine nutrition. Benefits of fat supplementation include meeting high energy requirements of performance horses and improving their performance (Nascimento de Godoi et al., 2010), maintaining body condition score (BCS), reducing the activity and reactivity of horses (Holland et al., 1996) and inducing metabolic adaptations that increase fat oxidation during exercise (Dunnett et al., 2002). Another important function of lipid is the absorption of fat-soluble vitamins and providing a source of linoleic acid, which is an essential fatty acid (Ellis and Hill, 2005).

Due to palatability, vegetable fats are more commonly fed to horses than animal fats. In their study with several different animal and vegetable fats, Holland et al. (1998) determined corn oil to be most easily accepted by horses in their diet.

Fats may supplement the horse's diet, since even though they are generally more expensive, their energy content is generally twice or more than that of available processed cereal grains (Potter et al., 1992; Ellis and Hill, 2005). In practice, hay or grass diets of horses are commonly supplemented with grain concentrates in order to meet energy requirements. This involves the risk of various digestive and metabolic disorders, like colic, laminitis, and insulin resistance (Pass et al., 1998; Treiber et al., 2005; Hoffman, 2009). Holland et al. (1998) suggested that these risks may be reduced by replacing carbohydrate supplements with fats or oils. This statement is controversial, since more recently, it has been demonstrated that increased adipose tissue and increased circulating fatty acids and triglycerides tend to increase insulin resistance (Lewis et al., 2002; Yu et al., 2002).

Psyllium supplementation in humans has been shown by Rodrigues-Moran et al. (1998) to result in a significant reduction of plasma triglycerides as well as glucose concentrations, even though no weight loss or reduced food intake was observed. This might be beneficial in the management of diabetes type 2 patients, in whom hypertriglyceridemia is most frequent (Rodrigues-Moran et al., 1998).

If psyllium would decrease the absorption of fat in the intestine of horses, and lower blood triglyceride concentrations, then energy requirements of performance horses supplemented with psyllium might not be achieved. On the other hand, it could help

horses that suffer from overweight and insulin resistance and could be a useful supplement to prevent and manage those conditions.

Water Intake

Water intake of the horse is determined by the effort to equalize body water loss, which can occur by four different routes: fecal, urinary, respiratory and cutaneous losses. In lactating mares there is also a loss through milk secretion (NRC, 2007). Water intake of horses is dependent on diet, ambient temperature, level of activity, and individual animal (NRC, 2007; Freeman, 1999). Water intakes are estimated to range from 5 to 8.4 L per 100 kg of BW; with greater requirements at higher temperatures and higher water intake on a hay only diet compared with grain supplementation only (NRC, 2007).

In a study by Danielson et al. (1995), horses fed 5.8 kg of a hay-only diet consumed 17.8 kg of water compared to 10.1 kg of water consumed by horses fed 1.8 kg of grain plus 1.3 kg of hay. These differences in water intake were explained by differing dry matter intakes, as well as different dietary compositions. Another study indicated that fiber intake affects water requirements (Pagan et al., 1999). They observed that in Thoroughbred horses a meal of 2.27 kg of hay resulted in elevated total plasma protein and greater water intake than a similar-sized meal of grain.

The primary anatomical site of water and electrolyte uptake is the unsacculated right dorsal colon (1 m), which is the largest colon in diameter, reaching approximately 30 to 35 cm, and also serves as the last site for absorption of nutrients (Moore et al., 2001).

Indicators of Hydration State

The main indicators of the hydration state of the horse can be measured as serum electrolytes (sodium, potassium, and chloride), packed cell volume (PCV) and plasma protein, which is an indirect measure of decreased plasma volume (Sloet van Oltruitenborgh-Oosterbaan, 1991; Pagan et al., 1999). Packed cell volume and total protein concentrations increase with the loss of fluid (Sloet van Oltruitenborgh-Oosterbaan, 1999; Cinotti et al., 1997).

Water intake and electrolyte concentrations of roughage consumed by horses might not be sufficient for an exercising horse, due to water loss as sweat and thermoregulation mechanisms (Coenen and Vervuert, 2003). This applies especially to performance horses, e.g. endurance horses, with high sweat losses (Grosskopf and Rensburg, 1983; Cinotti et al., 1997; Cuenen and Vervuert, 2003). Grosskopf and Renburg (1983) found that horses that show great variations in plasma protein and plasma calcium, magnesium and phosphate, pre- and post-endurance ride, are more likely to be disqualified before finishing the race than horses that maintain fairly stable concentrations of these variables. Therefore, improving or maintaining a stable hydration state is an important factor in some areas of the equine industry.

Sodium is an electrolyte that is of major importance, since it also assists with glucose and AA absorption in the intestine. Glucose and galactose are transported in the equine intestine by Na+/glucose co-transporter isoform 1 (Dyer et al., 2002). Amino acid transport also appears to be dependent on sodium. Al-Saleh and Wheeler (1981) found that the human erythrocyte membrane includes Na+ -dependent transport systems for

several AA. Mammalian reticulocytes have been shown to use a Na+ -dependent uptake mechanism for glycine and alanine (Youngs et al., 1976; Christensen and Hanlogden, 1981).

In their study in 1997, Cinotti et al. found that horses that had been supplemented with psyllium, showed significantly lower PCV's than control supplemented horses. This might be due to the water-holding capacity and decreased passage rate caused by psyllium supplementation, at least in the bovine (Cannon et al., 2010). Cinotti et al. (1997) suggested that increased water availability in the gut, together with the slower passage rate induced by psyllium, allows the intestine to absorb more fluid. If this is the case, psyllium supplementation might provide an important tool to minimize effects of dehydration and improve performance in horses.

CHAPTER 3

STATEMENT OF THE PROBLEM

Sand impaction has been known to be a cause for colic in horses in various geographic areas of the United States for a long time (Kaneene et al., 1996; Udenberg, 1979). Consumption of sand and dirt can lead to diarrhea, weight loss, and colic in horses (Hotwagner and Iben, 2008). Severe cases of sand colic require surgery, and, although long-term survival after surgery for sand colic can be as high as 95% (Granot et al., 2008), it is an expensive procedure. To avoid risks of sand colic and the resulting expensive treatment, it is a common practice to supplement horses with psyllium which is thought to assist in passage of ingested sand through the intestine of horses (Landes et al., 2008; Hotwagner and Iben, 2008).

Laminitis is a painful condition that is characterized by the hoof wall becoming detached from the underlying tissue. This can become so severe that the hoof will no longer support the animal's weight, resulting in a crippled horse (Sillence et al., 2007). It has been recommended that to prevent laminitis, glucose tolerance needs to be restored and insulin should be maintained at normal concentrations (Sillence et al., 2007). The most effective method to do this is to reduce the blood glucose concentrations to increase insulin sensitivity (RIRDC, 2010). According to recent research, one way to accomplish this might be to supplement horses with psyllium (Moreaux et al., 2011).

Psyllium supplementation decreased triglyceride concentrations in humans (Rodriguez-Moran et al., 1998) and glucose concentrations in humans and horses

(Rodriguez-Moran et al., 1998; Moreaux et al., 2011), but the effect of psyllium supplementation on concentrations of other nutrients has not been studied. Thus, the goal of this study was to identify the effects of psyllium supplementation on serum concentrations of protein and triglycerides as well as on the total feed intake in horses. Lower blood concentrations of these nutrients could indicate a decreased absorption of them through the intestine. If psyllium reduces absorption of important nutrients, then supplementation with it should be thoroughly considered, since nutrient deficiencies might result.

Furthermore, psyllium supplementation might improve the hydration state of horses. Increased water availability in the gut, together with the slower passage rate appears to allow the intestine to absorb more fluid and improve water balance (Cinotti et al., 1997). However, more research is necessary to confirm this concept. Thus, an additional goal of this study was to determine the effects of psyllium supplementation on serum electrolytes, PCV, and total water intake in horses.

CHAPTER 4

MATERIALS AND METHODS

Objective and Hypotheses

The objective of the study was to determine the effects of supplemental psyllium
fed to mature, light breed stock horses on serum protein, triglyceride, and electrolyte
concentrations and on packed cell volume (PCV), grazing intake, and water intake. The
hypotheses tested in this experiment were that serum protein, triglyceride and electrolyte
concentrations and PCV, as well as water and forage intake, did not differ between horses
supplemented with or without psyllium.

Horse Selection

Procedures were approved by the Montana State University Institutional Animal
Care and Use Committee (Protocol #2011-AA05). Eleven (7 mares, 4 geldings) 10- to18-
yr-old Quarter Horses were used in the study. All horses were selected from the Montana
State University Equitation Herd. Horses were first stratified by sex and then randomly
assigned to one of two treatments.

Treatments

There was a total acclimation time of 20 d to the diet of growing cool season
pasture grass. Grazing time was 3 h on the first d of grazing and increased by 1 h per d
until a total of 8 h was reached on d 5 of acclimation. The nutrient analysis of the

available forage is given in Table 4.1. All horses were individually fed 1 kg of a protein supplement (Nutrena Empower™ Balance Grass Formula) daily for the last 14 d of acclimation. The first 18 d of acclimation on pasture took place in a group setting; during the last 3 d strip grazing using electric tape was applied to isolate each horse. The treatments were: 180 g psyllium in pelleted form, 3.5 DE, Mcal/kg mixed with 453.6 g protein supplement (Nutrena Empower™ Balance Grass Formula), or 1,000 g of an isocaloric protein control supplement (Nutrena Empower™ Balance Grass Formula). Each horse received 90 g of oats to encourage psyllium intake. The resulting caloric intake was 1.62 Mcal. Nutrient composition of the protein supplement, oats and psyllium can be seen in Table 4.2. The amount of psyllium fed to each horse was based on that reported by Moreaux et al. (2011). Treatments were administered June 5, 2012 to July 3, 2012. Horses were allowed ad libitum access to clean water and a plain, white salt block. Psyllium refusals were recorded and are presented in the Results section (Table 5.1). Horses were housed in individual 3 x 4 m dry lots with shelter with an 8 h turn out period on 8.55 ha pasture with approximately 1.61 ha per horse. Strip grazing with electric tape was used to decrease the effects of herd behavior on grazing pattern and intake.

Table 4.1. Feed analyses of available forage on different collection days throughout the study

Item	Collection 1[1]	Collection 2[2]	Collection 3[3]	Collection 4[4]
DM, %	96.00	96.99	93.39	94.32
CP, %	19.23	13.51	12.46	9.39
NDF, %	53.82	57.67	58.87	61.98
ADF, %	23.91	30.54	33.34	38.28
Ash, %	58.14	11.12	12.26	12.45
Crude Fat, %	4.24	3.46	3.31	3.25
DE, Mcal/kg DM	1.96	1.94	1.95	1.88

[1]5/15/12 - Composite forage collection before begin of study
[2]6/4/12 - Average of all individual pens
[3]6/17/12 - Average of all individual pens
[4]7/1/12 - Average of all individual pens

Table 4.2. Feed analyses of protein supplement[1], oats, and psyllium

Item	Protein Supplement[1]	Oats	Psyllium
DM, %	92.95	91.61	93.09
CP, %	29.78	13.97	9.08
NDF, %	14.83	30.34	85.84
ADF, %	36.62	16.15	11.72
Ash, %	17.49	2.61	3.00
Crude Fat, %	4.57	3.65	3.15
NSC, %	33.33	49.43	0.00
DE, Mcal/kg DM	1.62	1.08	2.33

[1]Nutrena Empower™ Balance Grass Formula

Data Collection

Blood samples were collected for determination of serum electrolytes, serum triglycerides, serum protein and PCV. Water intake was determined by using metered water containers and was recorded twice daily for each horse. Total DM intake was determined by using the following equation that was previously published by Dowler and Siciliano (2009): 0.166 ± 0.015 kg DM $\times$ 100 kg BW^{-1} $\times$ h^{-1}. Body weight of the horses was determined at d 0 and d 29 of the study using an electronic scale (TruTest AG500, Mineral Wells, Texas). Body weights of horses in each treatment for these days are shown in Table 4.3.

For blood collections, each horse received an indwelling, jugular catheter the day before the collection day. On d 0, 8, 15, 22 and 29 blood samples were collected from each horse at 1500 h. Horses were kept individually on pasture, in their assigned grazing strip, and were undisturbed except for blood collection.

Forage clippings to determine availability and nutrient composition of forage took place before the beginning of the data collection period and every other week during the study.

Table 4.3. Mean BW of control- and psyllium-supplemented horses on day 0 and day 29 of the study

Item		Treatment			
		Control (n = 5)		Psyllium (n = 6)	
	Day	0	29	0	29
Average BW in kg		1220.2	1238.8	1167.0	1195.7

Sample Analyses

Analyses of forage availability and nutrient composition for determination of strip grazing allotment was conducted by clipping forage within ten 50 x 50 cm randomly located quadrats. Clipped samples were separated by species and dried in a forced air oven at 55° C for 48 h and then weighed and converted to kg/ha. The percentage of each plant species was also determined according to methods described by Smith et al. (1963) and Holechek et al. (1982). During the data collection period, forage availability was determined in samples collected from clipping three 25 x 25 cm quadrats that were located on a transect which was run in every grazing strip. Forage samples were ground with a Wiley Mill to pass a 1mm screen and stored for later analyses. The % NDF and ADF were determined in the manner determined by Van Soest (1991). Nitrogen was determined (LECO FP 528 N analyzer) and converted to CP (%) by multiplying by 6.25 (AOAC, 2012).

Blood samples for total protein, lysine, arginine, and total triglycerides were collected into Monoject™ tubes (Tyco Healthcare Group LP; Mansfield, Massachusetts, USA), without additive. Within 1 min of collection, tubes were put on ice. Samples were cooled at 4° C for at least 4 h and then centrifuged (1600 x g) for 30 min at 4° C. Serum was decanted into 12 x 75 mm plastic culture tubes and stored at -22° C.

Protein analysis was performed in duplicate using the BCA Assay (Thermo Scientific Pierce, BCA Protein Assay Kit, Thermo Fisher Scientific, Inc., Rockford, IL, USA). The method was evaluated using a pooled equine serum sample that was diluted with PBS (phosphate buffered saline) to the dilutions 1:400, 1:800, and 1:1600. The %

recovery associated with those dilutions was 104.6%, 100.2%, and 95.7%, respectively.

For all assays serum samples were diluted 1:400 with PBS. The intra- and inter-assay CV

were 6.0 and 2.0 %, respectively, for a serum sample that contained a mean of 183.6

µg/ml total protein. Another serum sample contained a mean of 203.3 µg/ml total protein

and the intra- and inter-assay CV for this sample were 4.0 and 3.0 %, respectively.

Total serum triglycerides were determined in duplicate using a serum triglyceride

kit (Cayman Chemical, Ann Arbor, MI, USA). This method was evaluated by using a

undiluted equine serum sample and the same sample diluted 1:2 with PBS. The %

recovery was 91.3% and 110.0%, respectively. The intra- and inter-assay CV were 5%

and 4%, respectively, for a standard that contained a mean of 102.6 µg/ml total

triglycerides. The low standard contained a mean of 25.7 µg/ml total triglycerides and the

intra- and inter-assay CV were 3.0% and 8.0%, respectively.

Serum samples for lysine and arginine were analyzed in the Department of

Chemistry and Biochemistry (Montana State University), using mass spectrometry

methods. The error associated with the machine was at 12%.

Blood samples for serum electrolytes were collected into Monoject™ tubes (Tyco

Healthcare Group LP; Mansfield, Massachusetts, USA). These samples were placed on

ice within 1 min of collection and then immediately taken to the Montana Veterinary

Diagnostic Laboratory (MVDL) for analyses. The machine used was a Siemens

Dimension Expand Plus. For sodium the intra- and inter-assay CV were 0.5% and 1.5%

for a low control, respectively, and 0.6% and 1.0% for a high control, respectively. The

intra- and inter-assay CV for potassium were 0.6% and 1.3% for a low control,

respectively, and 0.8% and 1.2% for a high control, respectively. For chloride, the intra-

and inter-assay CV were 0.6% and 1.4% for the low control, respectively, and 0.6% and

1.0% for the high control, respectively.

A blood sample for PCV was collected into Monoject™ tubes (Tyco Healthcare

Group LP; Mansfield, Massachusetts, USA) without additives, put on ice immediately

and analyzed on site using the standard microhematocrit method (centrifuge used: LW

Scientific ZIPocrit Hematocrit Centrifuge - model LWZIP2) according to Schalm et al.

(1975).

Statistical Analyses

The statistical software "R" was used to analyze protein, triglyceride, and serum

electrolyte concentration, and for PCV, water and forage intake. The experimental unit

was individual horse. There were 6 experimental units in the psyllium-supplemented

treatment and 5 experimental units in the control-supplemented treatment. At first, the

'full' model (one that included all potential explanatory variables) was evaluated. The

explanatory variables were: treatment (psyllium/control), sex (gelding/mare), age, forage

intake, water intake, collection day, and the interaction between treatment and collection

day. A repeated measures ANOVA model with a compound symmetric correlation

structure was fit and continued with a stepwise model reduction procedure. The repeated

measure was collection day. Least squares means were generated for each variable. At

each step, the variable with the largest P-value > 0.05 was dropped and the model was

refit. This process was continued until all variables had P-values < 0.05. Means were

separated using the Bonferroni Multiple Comparison Method. Means will be presented

and associated SE will be used throughout the results.

CHAPTER 5

RESULTS

Protein

There was no interaction between the independent variables ($P > 0.05$). However, psyllium supplemented horses had lower ($P = 0.002$) serum protein concentrations than control-supplemented horses. Mean serum protein concentration for all collection d was 7.4 ± 0.2 g/dL for control-supplemented horses and 7.2 ± 0.1 g/dL for psyllium-supplemented horses. Based on these findings, it can be concluded that psyllium supplementation decreased serum protein concentration by 0.2 g/dl for horses of similar ages. Mean serum protein concentrations on each collection day for control- and psyllium-supplemented horses are shown in Table 5.1.

Protein concentrations of mares were lower ($P = 0.001$) than those of geldings throughout the study. Mean serum protein concentration for control-supplemented horses for all collection d was 6.6 ± 0.5 g/dL and. 7.9 ± 0.18 g/dL for mares and geldings, respectively. For psyllium-supplemented horses, mean serum protein concentration for all collection days was 7.1 ± 0.17 g/dL and 7.3 ± 0.07 g/dL for mares and geldings, respectively.

Lysine

There was no effect of psyllium supplementation on serum lysine concentrations ($P = 0.110$). Mean serum lysine concentration for all collection d was 11.8 ± 0.5 ng/mL

for control-supplemented and 14.1 ± 0.7 ng/mL for psyllium-supplemented horses. Mean

serum lysine concentrations on each collection d for control- and psyllium-supplemented

horses are shown in Table 5.1. While the original analysis indicated a significant effect

for time ($P = 0.016$), there was no significance after adjusting the P - Values with the

Bonferroni Multiple Comparison Method. When comparing all collection days to

another, all P – values were > 0.123.

Arginine

There was no treatment ($P = 0.069$) or treatment by time ($P = 0.086$) interaction

for serum arginine concentrations. Mean serum arginine concentrations on each

collection d for control- and psyllium-supplemented horses are shown in Table 5.1. Mean

arginine concentration throughout the study was 16.1 ± 1.2 ng/mL for control-

supplemented horses, and 20.7 ± 0.5 ng/mL for psyllium-supplemented horses.

Triglycerides

There was no interaction ($P > 0.05$) between the independent variables. However,

psyllium-supplemented horses showed reduced concentrations of triglycerides ($P = $

0.003) compared to control-supplemented horses (Table 5.2). Mean serum triglyceride

concentration for all collection days was 38.2 ± 0.4 µg/mL for control-supplemented

horses and 35.5 ± 0.8 µg/mL for psyllium-supplemented horses.

Table 5.1. Mean serum total protein, lysine, and arginine concentrations of control- and psyllium-supplemented horses on the different collection d of the study

Item		Treatment									P - Value		
		Control (n = 5)				Psyllium (n = 6)							
	Day	8	15	22	29	8	15	22	29	SE	trt	time	trt*time
Serum total protein (g/dl)		6.8[a]	7.5[a]	7.5[a]	7.7[a]	7.0[b]	7.0[b]	7.5[b]	7.2[b]	0.15	0.019	0.337	0.645
Serum lysine (µg/ml)		12.8	1.9	12.1	10.5	14.9	15.6	14.0	12.2	0.65	0.110	> 0.123	0.446
Serum arginine (µg/ml)		18.3	2.8	15.7	17.4	21.9	21.1	19.9	19.8	0.90	0.069	0.086	0.097

* Serum total protein concentrations marked with the letter [a] are statistically different ($P < 0.05$) from those marked with the letter [b]

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Table 5.2. Mean serum triglyceride concentrations of control- and psyllium-supplemented horses on the different collection d
of the study

Item	Control (n = 5)				Psyllium (n = 6)				SE	P - Value		
		Treatment										
Day	8	15	22	29	8	15	22	29		trt	time	trt*time
Serum triglycerides (µg/ml)	37.7[a]	37.5[a]	39.5[a]	38.2[a]	29.9[b]	33.6[b]	32.0[b]	31.1[b]	0.60	0.001	0.976	0.989

* Serum total triglyceride concentrations marked with the letter [a] are statistically different ($P < 0.05$) from those marked with
the letter [b]

Packed Cell Volume

There was no treatment ($P = 0.991$) or treatment by time ($P = 0.268$) interaction for PCV's. Mean PCV for all collection days was $40.61 \pm 0.7\%$ for control-supplemented horses and $40.68 \pm 0.7\%$ for psyllium-supplemented horses. Mean PCV's on each collection day for control- and treatment-supplemented horses are shown in Table 5.3. There was a significant ($P < 0.001$) effect of time on PCV. Mean PCV's on collection day 0, 8, and 29 were significantly lower than on day 15, and 22 (Table 5.3).

Serum Electrolytes

Sodium

There was no treatment ($P = 0.089$) or treatment by time ($P = 0.486$) interaction for serum sodium concentrations. Mean sodium concentration for all collection days was 136.9 ± 0.6 mmol/L for control-supplemented horses and 136.3 ± 0.7 mmol/L for psyllium-supplemented horses. There was a significant ($P < 0.001$) effect of time on sodium concentration (Table 5.3). A significant difference in sodium concentrations could be detected between d 0 and all other collection days, as well as between d 22 and day 15 and 8. The mean sodium concentration on each collection day for control- and psyllium-supplemented horses is shown in Table 5.3.

Potassium

There was a treatment by time ($P = 0.032$) interaction for serum potassium concentrations. Psyllium-supplemented horses had significantly higher serum potassium

concentrations on collection d 8 ($P = 0.003$) and on d 22 ($P = 0.673$). On d 0 ($P = 1$), day 15 ($P = 0.673$), and d 29 ($P = 0.009$), potassium concentrations were not different in control-supplemented compared to psyllium-supplemented horses. Mean potassium concentrations on each collection day for control- and treatment- supplemented horses are shown in Figure 5.1.

Chloride

There was no treatment ($P = 0.514$) or treatment by time interaction ($P = 0.371$) for serum chloride concentrations. Mean chloride concentration for all collection days was 101.72 ± 0.5 mmol/L for control-supplemented horses and 102.00 ± 0.7 mmol/L for psyllium-supplemented horses. There was a significant ($P < 0.001$) effect of time on chloride concentrations. Chloride concentrations on d 0 were greater than on all other collection d. Also, chloride concentration on d 8 was greater than that on d 29. Mean chloride concentrations on each collection day for control- and treatment- supplemented horses are shown in Table 5.3.
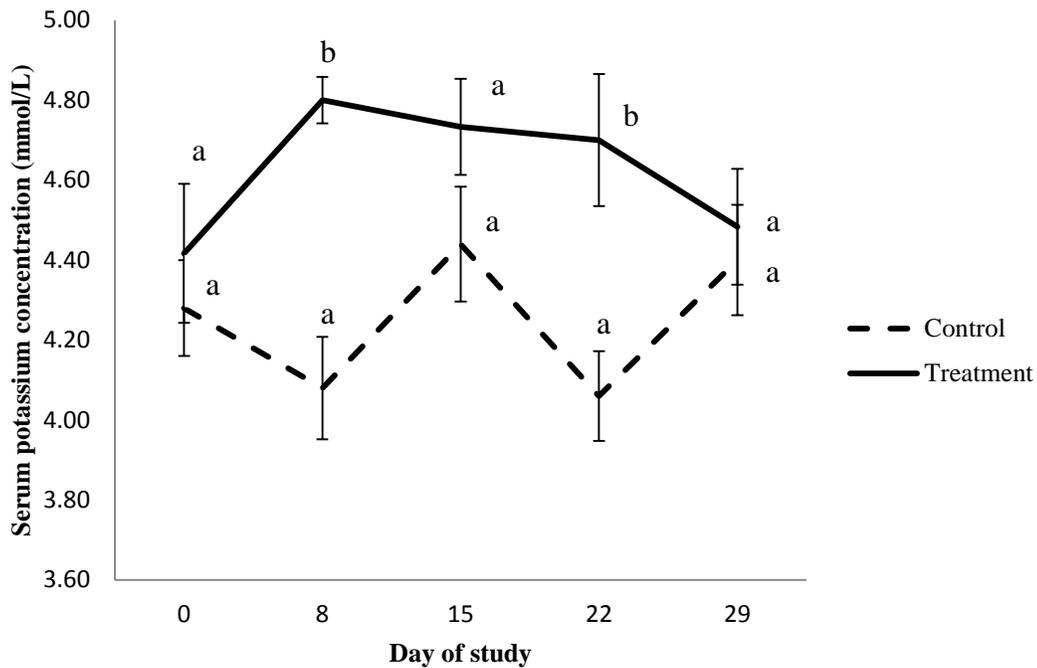
Figure 5.1. Mean serum potassium concentrations of control- and psyllium-supplemented horses on the different collection d of the study. Potassium concentrations on data points marked with the letter "a" are statistically different from those marked with a "b".

Water Intake

There was no treatment ($P = 0.193$) or treatment by time ($P = 0.377$) interaction for the total water intake per horse/d. The mean water intake throughout the study was 21.2 ± 1.8 L per day for control-supplemented horses and 23.8 ± 1.9 L per d for psyllium-supplemented horses. However, there was a significant effect of time on water intake ($P < 0.001$). Water intake increased by on average 0.25 L per d throughout the study. Mean water intake for control- and psyllium-supplemented horses throughout the study are given in Figure 5.2.

Table 5.3. Mean PCV's and sodium and chloride concentrations of control- and psyllium-supplemented horses on the different collection d of the study

	Treatment											*P* - Value		
Item	Control (n = 5)					Psyllium (n = 6)								
Day	0	8	15	22	29	0	8	15	22	29	SE	trt	time	trt*time
PCV (%)	39.8^a	40.5^a	43.0^b	41.0^a	38.8^a	39.8^a	38.8^a	42.3^b	42.5^b	40.0^a	0.70	0.991	< 0.001	0.268
Sodium (mmol/l)	138.6^a	137.2^b	137.0^b	135.0^b	136.6^c	139.0^a	136.2^b	136.3^b	134.8^b	136.6^c	0.65	0.089	< 0.001	0.486
Chloride (mmol/l)	103.6^a	102.0^b	101.2^b	100.8^c	101.0^b	104.3^a	102.5^b	101.0^b	101.7^c	100.5^b	0.60	0.514	< 0.001	0.371

* The letters [a], [b], and [c] indicate a statistical difference ($P < 0.05$) in PCV, and sodium and chloride concentrations on the different collection days
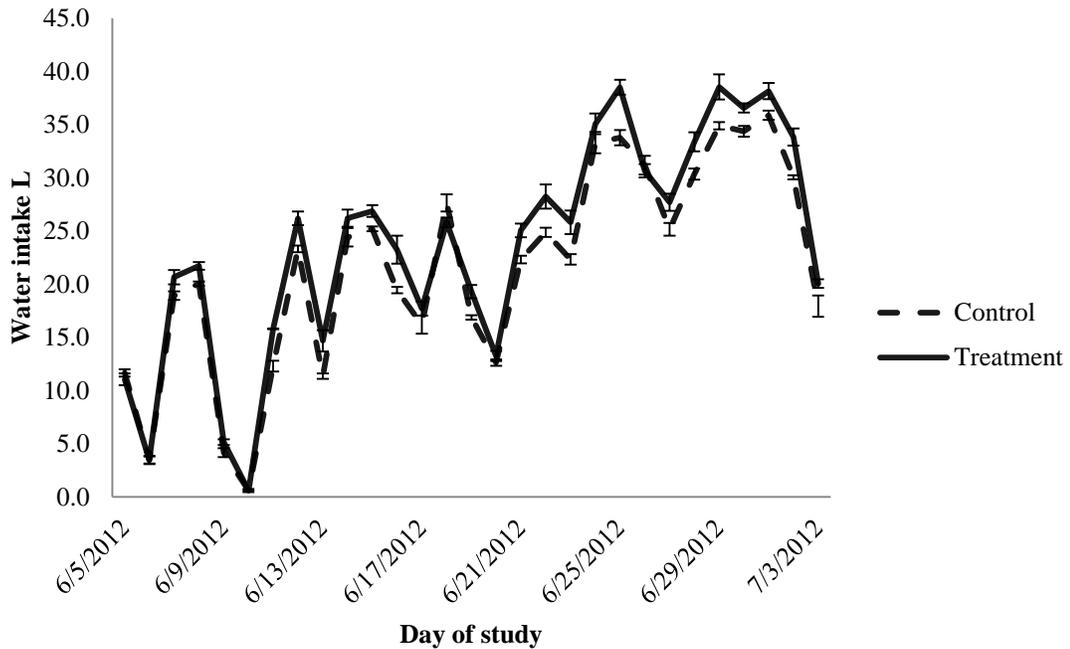
Figure 5.2. Mean water intake of control- and psyllium-supplemented horses on the different collection d of the study.

Forage Intake

No treatment ($P = 0.074$) or treatment by time interaction ($P = 0.051$) was detected for the forage intake. However, there was a significant effect of time ($P < 0.001$) on forage intake. The mean forage intake increased with an increase in time. The mean forage intake of control- and psyllium-supplemented horses can be seen in Table 5.4.

Table 5.4. Mean forage intake of control- and psyllium-supplemented horses on the different collection days of the study

Item	Control (n = 5)					Psyllium (n = 6)					SE	P - Value		
Day	0	8	15	22	29	0	8	15	22	29	SE	trt	time	trt*time
Forage Intake (kg)	16.2^a	16.3^b	16.3^c	16.4^d	16.5^e	15.5^a	15.6^b	15.7^c	15.8^d	15.9^e	0.06	0.074	< 0.001	0.051

* The letters [a], [b], [c], [d], and [e] indicate a statistical difference ($P < 0.05$) in Forage Intake on the different collection days

Psyllium Refusals

Not all psyllium was consumed by all horses every day. If there were psyllium refusals in the morning, the remaining pellets were left in the feed pen in the dry lot, to give the horse the chance to consume it when turned in from pasture and overnight. If consumption overnight did not occur, the refusal was weighed and disposed of. The psyllium refusals of individual horses are reported in Table 5.3.

Table 5.5. Psyllium refusals of horses throughout the study.

Horse ID	Date	Quantity
13	6/12/2012	20 g
13	6/16/2012	15 g
13	6/17/2012	20 g
12	6/17/2012	10 g
13	6/19/2012	2.61 g
13	6/20/2012	105 g
13	6/22/2012	Finished overnight
13	6/23/2012	Finished overnight
13	6/24/2012	Finished overnight
13	6/25/2012	Finished overnight
17	6/25/2012	Finished overnight
13	6/26/2012	Finished overnight

CHAPTER 6

DISCUSSION

Protein

Based on our findings it can be concluded that psyllium supplementation decreased serum protein concentration by 0.2 g/dl for horses of similar ages. Cinotti et al. (1997) supplemented horses with psyllium for 8 d and measured total serum protein. They did not find a significant difference in horses receiving psyllium compared to those that did not, which is in contrast to the results of the present study. The reason for the disparate results might be that the study by Cinotti et al. (1997) was only 1 wk long, with 1 blood collection d. The results regarding protein concentration of the present study suggest that a study of only 1 wk might not be long enough to detect differences in protein concentrations in supplemented horses. While no other study has previously evaluated the effects of psyllium on absorption of protein, it has previously been found that the gel-like substance formed by psyllium aids passage of ingested sand and dirt in the hindgut of horses (Landes et al., 2008; Hotwagner and Iben, 2008). Also, it has been found that psyllium decreased glucose concentrations in humans and horses, as well as triglyceride concentrations in humans (Rodrigues-Moran et al., 1998; Blackwood et al., 2000; Moreaux et al., 2011). Based on those findings, it could be suggested that the mucilage of psyllium might propel feed particles through the intestine and prevent digestion/absorption of nutrients contained in the intestinal content.

Lysine and Arginine

The results of the present study indicate that psyllium-supplementation may lead to decreased serum protein concentration in horses. However, it is important to understand what AA psyllium is affecting in order to determine effects that psyllium supplementation has in horses. Therefore, in this study the two essential AA lysine and arginine, of which lysine is the first limiting AA in horses, were analyzed. The results indicated that there was no significant effect of psyllium on lysine and arginine concentrations, suggesting that those two AA are not affected by psyllium supplementation. However, visual assessment of these results suggests that further research is necessary to better understand effects of psyllium-supplementation on concentrations of AA. The exact lysine and arginine content of psyllium would need to be investigated to further interpret these results. Numerically higher concentrations of lysine and arginine in psyllium-supplemented horses may reflect lysine and arginine content of psyllium. This idea remains to be investigated.

<div align="center">Triglycerides</div>

Results of this study regarding the effect of psyllium-supplementation on serum triglyceride concentrations are similar to those in human research. Psyllium-supplemented horses in the present study showed lower serum triglyceride concentrations than control-supplemented horses. Similar results have been reported by Rodrigues-Moran et al. (1998), wherein triglyceride concentrations were lowered in humans that received psyllium supplementation compared with those that did not receive a

supplement. These findings would again suggest that the mucilage of psyllium hinders absorption/digestion of nutrients in the intestine in humans and horses.

PCV

Psyllium supplementation did not effect PCV of horses in the present study. These results are in contrast with those reported by Cinotti et al. (1997), wherein PCV in horses that received 0.3 g/kg BW psyllium remained significantly lower throughout an endurance race than in horses that did not receive psyllium. However, it is questionable as to whether the results reported by these authors compare with those of the current study. The different design of these two studies might have led to differing results. In the study by Cinotti et al. (1997), horses were exercised in an endurance race, while in this study horses were not exercised. The variability of equine PCV appears to be related to the type and intensity of exercise performed, the state of training, acclimatization and the state of hydration of the horse. More studies need to be done in order to determine when and if psyllium can affect PCV in horses.

Serum Electrolytes

While psyllium-supplementation did not have an effect on serum sodium and chloride concentrations, it appeared to have an effect on serum potassium concentrations on d 8, and 22 after the start of treatment. The results for sodium and chloride coincide with previous findings in a study with horses during an endurance race by Cinotti et al. (1997). The fact that no significant differences could be found in serum electrolytes, and

all values remained almost within the normal range lead Cinotti et al. (1997) to the

assumption that none of the horses had serious problems regarding electrolyte balance.

This can also be assumed for sodium and chloride balance in horses of the present study,

where all concentrations remained within the normal range.

The downward trend of sodium and chloride concentrations throughout the study

might have been caused by the increasing water intake that resulted from increasing

temperatures throughout the study. Consequently, there might have been an increase in

the amount of body water relative to sodium and chloride, which resulted in lowered

sodium and chloride concentrations. In contrast to the results of this study, Cinotti et al.

(1997) did not find a difference in potassium concentrations between psyllium- and

control-supplemented horses. Cinotti et al. (1997) reported potassium concentrations of

4.1 mmol/L and 4.0 mmol/L for psyllium- and control-supplemented horses, respectively,

before starting an endurance race. These concentrations are almost in the normal range

for horses, which is 3 to 4 mmol/L.

Potassium concentrations for horses in the present study were slightly elevated

with an average of 4.3 mmol/L and 4.6 mmol/L for control- and psyllium-supplemented

horses, respectively. Elevated potassium values have been reported to occur during high

intensity exercise as a consequence of efflux of potassium from the active muscle (Harris

and Snow, 1992). Another reason for elevated potassium levels can be "hyperkalemik

periodic paralysis" (HYPP), a genetic disease linked to the Quarter Horse stallion

"Impressive". This disease results in a failure of a subpopulation of sodium channels to

inactivate when serum-potassium concentrations are increased. The major clinical sign of

this disease is fatigue (Spier, 2006). However, none of the horses in the present study underwent intense exercise, were genetically linked to the stallion "Impressive", or showed signs of fatigue. The most likely reason for the slightly elevated appearance of potassium concentrations is that serum was used for the analysis, instead of plasma. Due to the release of potassium from platelets during clotting, serum potassium is higher than plasma potassium (Hartland and Neary, 1999).

The very similar potassium values on d 29 of the present study could indicate an adaptation of the intestinal microflora to the psyllium, which eliminates the effect of psyllium. The idea of adaptation to psyllium, which leads to an improved ability to degrade it in the hindgut, has also been suggested in previous studies (Campbell and Fahey, 1997; Hammock et al., 1998).

Elevated potassium concentrations in this study could be an indicator of the water-binding capacity and the decreased passage rate that psyllium is thought to cause. Thus, there is the possibility that psyllium caused an increase in intestinal absorption of water and therefore improved the hydration state of the horses. However, this effect could be attenuated once microflora in the hindgut of horses adapt to psyllium. Due to the small sample size used in the present study and the fact that psyllium pellets and the protein supplement were not analyzed for potassium concentrations it is difficult to declare that the results of this study were related to microflora adaptation. Additionally, psyllium is known to contain significant amounts of potassium (Powell et al., 1982). Therefore, it is possible that horses that received psyllium supplement showed elevated potassium

concentrations for the reason that their potassium intake was greater. Further research is necessary to explore the effects of psyllium on potassium concentrations in the horse.

Water Intake

Psyllium supplementation did not influence water intake of horses in the present study. The increasing trend of water intake throughout the study may be explained by increasing temperatures and decreasing precipitation as well as water content of forage throughout the study.

Forage Intake

Psyllium supplementation did not influence forage intake in the present study. Although psyllium supplementation did not alter forage intake in terms of kg/DM, it was found that the forage intake increased significantly in all horses throughout the study. This could be due to decreasing nutrient contents in the forage available to each horse, which can be compensated for by increasing intake.

CHAPTER 7

CONCLUSION

Psyllium can be a useful supplement in horses, not only to treat and prevent sand colic, but also to treat and prevent equine metabolic syndrome (Hotwagner and Iben, 2008; Moreaux et al., 2011). Previous research indicated that psyllium has an effect on the absorption of nutrients in the intestine. Psyllium supplementation decreased glucose and triglyceride concentrations in humans and glucose concentrations in horses (Rodriguez-Morán et al., 1998; Moreaux et al., 2011). This can be beneficial for managing horses with equine metabolic syndrome. However, if psyllium also decreases absorption of other important nutrients, like protein, then supplementation might lead to nutrient deficiencies. In the equine industry it is common to feed protein and fat/oil supplements to horses, especially those that are assumed to have high requirements of those nutrients.

The results of the present study with respect to total protein and triglyceride concentrations support the aforementioned statement. Psyllium supplementation at 180 g/d decreased total serum protein and triglyceride concentrations in horses, which may indicate a decreased absorption of those nutrients in the intestine. However, the effects of psyllium on two essential AA, lysine and arginine might indicate that there was no effect on absorption of those particular AA. Psyllium supplementation did not decrease the serum concentrations of those AA in horses of the present study. Further research with a

larger study size and detailed AA analyses of psyllium needs to be performed in order to better understand those results.

Previous research indicated that psyllium supplementation lowered PCV in horses, which suggests an improved hydration state of the horse (Cinotti et al., 1997). In the present study psyllium supplementation did not influence PCV and two serum electrolytes, sodium and chloride. However, the psyllium appeared to increase serum potassium concentration on some of the sampling days in this study. This result could indicate an improved hydration state of the horses in the study. However, it could simply be due to the fact that psyllium contains significant amounts of potassium (Powell et al., 1982). Disparate results of the previous study by Cinotti et al. (1997) and the present study could be due to the different experimental designs and protocols. In the previous study (Cinotti et al., 1997), horses were exercised heavily during an endurance race, which is likely to change parameters of hydration; while horses in the present study, were not exercised and therefore did not have any difficulties maintaining a proper hydration state. Further research is necessary in order to determine the effects of psyllium supplementation on the hydration state of horses.

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