A COMPARISON OF RICE BRAN OIL AND CORN OIL IN THE EQUINE DIET

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ABSTRACT

Rice bran oil (RBO) has gained attention as a fat and potential antioxidant supplement. Limited research has addressed the effects of RBO in the equine diet. This study compared the effect of RBO versus corn oil (CO) on non-esterified fatty acids (NEFA) and triglyceride (TG) concentrations, rump fat (RF), forearm (ARM) and gaskin (GAS) circumference, and total antioxidant status (TAS) in lightly exercised horses. Twelve mature horses were randomly assigned to a cross-over design where they received either RBO or CO at a rate of 324 mg/kg BW/d for 35 days. After 5 weeks of supplementation, the horses were given a 3-week washout period with no oil supplement, followed by 35 days of the opposing treatment. During both periods, on days 0, 17, and 35 RF, GAS and ARM circumference, and BW were measured. Serum NEFA, TAS, and TG were analyzed through obtained blood samples. Data were analyzed using a mixed model with repeated measures in SAS. No differences were found in RBO vs CO in any variables measured, BW ($P > 0.11$), RF ($P>0.68$), GAS($P>0.70$), ARM ($P>0.33$), TG ($P>0.70$), NEFA ($P>0.46$), or TAS ($P>0.16$). The results from this study indicate that RBO has similar effects to CO when fed as a fat supplement to horses.

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FAT and ANTIOXIDANT SUPPLEMENTATION

Introduction

Horse owners commonly use fat in equine diets to provide different benefits to the horse. Supplementation of fat may improve the performance of the horse including impacts on reproductive activity, coat appearance, thermoregulation, and fuel utilization. Supplementation is sometimes used to provide access to essential fatty acids as well as increase caloric density, or the amount of energy provided in the diet. As oxidative stress is also a concern to the athletic horse, it is also reasonable to consider the influence of any supplements on markers of oxidative stress or the animal's antioxidant system. Studies have been conducted to enhance the understanding of specific physiologic responses caused by specific fats or oils, but little research today has considered antioxidant properties that some oils may possess. This review will examine research on fat supplementation, as well as the influence of other supplements on oxidative stress and antioxidant status in the horse.

Fat Supplementation

To provide an additional source of fat to the diet, fat is commonly supplemented through inclusion in commercial concentrate feeds or through the use of oils top-dressed to the ration. Oil supplementation provides a highly digestible source of lipids that play an important role in many metabolic pathways in the body (Crandell et al., 1999). Lipids can be used as a source of energy during skeletal muscle metabolism and provide a densely packed form of energy when these muscles are in use (Geelen et al., 2002).

Structure of Fats Fats have a triglyceride, or triacylglycerol, molecular structure, which consists of a glycerol backbone attached to three fatty acids (Garrido et al., 2015). The number of carbon atoms present and the placement of double bonds in the carbon chain determine the names of the fatty acids. The three categories of fatty acids are saturated, monounsaturated, and polyunsaturated (PUFA).

Saturated fatty acids do not have a double bond in the carbon chain, meaning their tales are straight, and they are solid at room temperature. For this reason, saturated fatty acids are not commonly used for fat supplementation in the equine diet. Monounsaturated fatty acids contain only one double bond in the carbon chain. Polyunsaturated fatty acids contain more than one double bonds in the carbon chain. Both mono- and poly-unsaturated fatty acids are liquid at room temperature because of the bend(s) in their chains. This makes them more ideal for fat supplementation.

Many of the oils commonly used for supplementation provide sources of the essential fatty acids (EFA) categorized as Omega-3 (such as alpha-linoleic acid, ALA) and Omega-6 (such as linoleic acid, LA) fatty acids, both named based on their placement of the double bond. Both Omega-3 and Omega-6 fatty acids are important in the equine diet because the horse cannot synthesize them naturally. Therefore, these fatty acids must be provided to the equine diet as fat supplementation, forages, or grains (O'Connor et al., 2004). However, their downstream metabolites vary, and research suggests Omega-6 fatty acids to be inflammatory than Omega 3 fatty acids, thus prompting additional research to identify the ideal oil source.

Digestion of Fat When the horse consumes fat, it is partly digested by the stomach via the activity of the digestive enzyme, gastric lipase. The majority of the fat is then broken down and absorbed by the duodenum of the small intestine (Doreau and Chilliard, 1997). Contractions produced by the small intestines allow for further break down of fat particles (Senior, 1964). As part of the digestive process, the liver continuously releases bile into the small intestine in order to emulsify the fat globules (Frape, 1998). In animals, other than the horse, bile is stored in and released from the gallbladder. However, the horse does not have a gallbladder, so the bile is released directly from the liver (Caylor, 1952). A lipase enzyme, which is released from the pancreas, attacks the emulsified fats in order to further breakdown and digest the fat molecules (Frank et al., 2004). This enzyme attacks triglycerides resulting in the release of two free fatty acids and a 2-monoglyceride which then form micelles (Doreau and Chilliard, 1997). The micelles are absorbed by intestinal mucosal cells through passive diffusion. The fatty acids and monoglycerides from the broken-down triglycerides are then reassembled in the endoplasmic reticulum. They are then packaged with cholesterol esters and phospholipids into chylomicrons, which are lipoproteins that act as carriers for triglycerides. These chylomicrons are then released into the lymphatic system allowing them to enter into the blood stream where they can be transported throughout the body.

Dietary Fat Forages and grains, which primarily contain starches, make up the diet commonly used for domesticated horses and provide the main source of energy. As these feedstuffs are naturally low in fat, the traditional equine diet may have dietary fat

concentration in a range of 3-5%. A high percentage of fat in the equine diet is between a range of 8-12% while the average fat intake for humans consists of 30% or higher (Kreider et al., 2010).

As horses do not commonly consume high levels of fat, adaptation to a fat supplemented diet must occur. Horses exhibit signs of adaptation to fat after five weeks of supplementation while being exercised regularly (Pagan et al., 2002). After 10 to 12 weeks of supplementation, horse have full metabolic adaptation to the fat supplement (Orme et al., 1997).

Oils, Omega-3, and Omega-6 Ratios Oils that are supplemented in the diet contain different ratios of omega-3 and omega-6 fatty acids. However, the optimal ratio for the horse is unknown. Oils that contain higher amounts of Omega-6 fatty acids include corn oil (53:1; Omega-6: Omega-3 respectively), rice bran oil (19:1), soybean oil (7:1), and canola oil (2:1) (Cleland et al., 2006). Flaxseed (1:3) and fish oil (1:10) contain higher amounts of omega-3 fatty acids (Cleland et al., 2006). Fish oil is also unique due to oil's high amounts of eicosapentaenoic acid (EPA) and docosachexaenoic acid (DHA), which can be derived from ALA but typically at a low conversion rate. EPA and DHA have been shown to reduce inflammation and may thin blood (Cleland et al., 2006).

Omega-3 and Omega-6 Fatty Acid Supplementation Omega-3 and Omega-6 supplementation may differentially influence lipid metabolic variables such as non-esterified fatty acids (NEFAs) and triglycerides. Triglycerides provide a source of fatty acids during muscle metabolism in the body (Monteverde et al., 2017). "NEFAs are used

as an indicator of the ratio between fatty acid mobilization from adipose tissue and fatty utilization in muscular tissue" (Monteverde et al., 2017).

A 2017 study examined the supplementation of polyunsaturated fatty acids to the equine diet by evaluating the lipid profile of the subjects (Monteverde et al., 2017). The supplement consisted of a combination of omega-3 fatty acids, DHA, and EPA (Monteverde et al., 2017). After a supplementation of four weeks and being in a racing program, the study found a decrease in the subjects' serum triglyceride and NEFA levels compared with the control group that received no supplementation (Monteverde et al., 2017). These results coincided with an earlier study that used the same supplement combination for four weeks with athletically active horses, including jumpers as well as racing Thoroughbreds (Piccione et al., 2014). These results indicate that Omega-3 supplementation increases the utilization of fat used for energy during exercise.

O'Connor et al. (2007) examined similar variables in Thoroughbreds by specifically supplementing fish oil (higher Omega-3) and corn oil (higher Omega-6) to the horses. The study found lower levels of serum triglyceride concentrations in the fish oil group compared to the corn oil group after 63 days of supplementation (O'Connor et al., 2007). The results for NEFAs did not correspond with the triglycerides, showing no differences in either group (O'Connor et al., 2007). The decrease in triglyceride concentrations can be an indication that Omega-3 fatty acids were efficiently utilized by the body. The lack of change in NEFA concentrations may be in relation to the varying ratios of fatty acids present in the diet (O'Connor et al., 2007). O'Connor et al. (2004) performed a similar study earlier but found no differences in the triglyceride

concentrations between the fish and corn oil groups. However, NEFA concentrations were not examined in the group of horses supplemented.

A 2005 examination of rice bran oil examined plasma NEFA and triglyceride concentrations after supplementation (Frank et al., 2005). The horses examined were supplemented with water, corn oil, or rice bran oil (Frank et al., 2005). After five weeks of supplementation, the results suggested a decrease in all the variables measured and oils supplemented (Frank et al., 2005). The decrease in triglycerides and NEFA concentrations relate to the utilization of fat used as an energy source for horses.

Antioxidant Supplementation

Oxidative stress is the imbalance between free radicals and antioxidants present in the body. Free radicals, or reactive oxygen species (ROS), are oxygen-containing molecules that are not reduced completely in the blood (Williams, 2016). These molecules can damage DNA, lipids, and proteins if not regulated correctly (Williams, 2016). Research involving rats, humans, and horses showed this connection to muscle damage in these researched populations (Dillard et al., 1987; Powers and Jackson, 2008). Providing an increase in antioxidants to their already existing supply could help balance the accumulation of ROS in the system and reduce the risk of damage to muscle tissue.

Natural Antioxidants The body can naturally protect itself from ROS accumulation by producing enzymes that help maintain a balance if there is a high level of oxidants (Kuhn, 2003). These enzymes, known as antioxidants, naturally react to ROS which neutralizes them. Some examples of these antioxidant enzymes are alpha lipoic acid (super

antioxidant), coenzyme Q10 (CoQ10), and glutathione (GSH) which are produced by the body (Kuhn, 2003). Kuhn (2003) stated that Vitamin E and Vitamin C are also common antioxidants used to help combat excessive ROS but are not naturally produced in the body. Vitamin E and Vitamin C can be provided in the diet instead (Kuhn, 2003). Researchers have found that Vitamin C and GSH are both antioxidants known to help regenerate other antioxidants making them especially importance in the body (Lykkesfeldt and Svendsen, 2007).

Alpha lipoic acid is one of the most powerful antioxidants because it is both fat- and water-soluble and has the ability to promote synergy among other antioxidants (Kuhn, 2003). Glutathione is water-soluble and the primary antioxidant of the cell. Glutathione is vital for liver function and for fighting infection (Kuhn, 2003). CoQ10 is a powerful fat-soluble, or lipophilic, antioxidant that provides protection and support of the mitochondria (Kuhn, 2003).

Antioxidant and Oxidative Stress Markers Oxidative stress is the imbalance between oxidants and antioxidants present in the body which causes damage. Oxidative damage measurements focus on DNA, proteins, and lipids during analysis (Lykkesfeldt and Svendsen, 2007). Damage to DNA related to oxidative stress can be measured either through tissue samples or the number of broken strands found in singular cells (Collins, 2004). Broken DNA strands are examined through methods of complex high-performance liquid chromatography (HPLC) or mass spectrometry (Lykkesfeldt and Svendsen, 2007).

Lipid oxidation is commonly measured through two assays: malondialdehyde (MDA) and isoprostane assays (Lykkesfeldt and Svendsen, 2007). Both assays are measured through mass spectrometry. Both markers have been found to be reliable when assaying for lipid peroxidation (Morrow, 2000). MDA is a breakdown product that measures the population of lipid hydroperoxides (LPO) which is in correlation to lipid peroxidation (Lykkesfeldt and Svendsen, 2007). MDA assays are most commonly used when examining oxidative damage due to the simplicity of the method.

Protein oxidation is a common measurement used in analysis because of its simplicity. The tests are conducted through means of colorimetric assays, but have been found to have more ambiguous data (Lykkesfeldt and Svendsen, 2007). Glutathione-peroxidase activity (GPx) and superoxide dismutase (SOD) are specific protein markers used to evaluate oxidative stress (Yavari et al., 2015). Glutathione-peroxidase is an enzyme that can be used when examine oxidative stress in the body, because it is produced to remove hydrogen peroxide and other organic hydroperoxides from the cell (Yavari et al., 2015). SOD is an enzyme that initiates the reduction of superoxide anions to hydrogen peroxide, which is less reactive (Yavari et al., 2015). The functions of these enzymes help regulate the number of oxygen molecules that are free in the cell which helps prevent possible damage to the cell.

Antioxidants can also be measured to see the effect of a supplement on antioxidant levels after supplementation. Multiple types of measures are used to determine the concentration of all antioxidants or of a specific antioxidant. Antioxidant status, also known as total antioxidant status (TAS), can be evaluated when examining oxidative stress by measuring the capacity of antioxidants (Lykkesfeldt and Svendsen,

2007). Similar measurements include Trolox equivalent antioxidant capacity (TEAC), total radical-trapping antioxidant parameter (TRAP), and ferric-reducing antioxidant power (FRAP) (Lykkesfeldt and Svendsen, 2007). These different types of analysis all examine the activity of all antioxidants and present it in a single value. GSH and ascorbate are more specific measurements of antioxidant biomarkers that can be measured through methods of HPLC (Lykkesfeldt and Svendsen, 2007). These overall variables are used to evaluate the effects of oxidative stress.

Vitamin E and C Various kinds of supplements are used in order to combat the negative effects of oxidative stress. Alpha-tocopherol (vitamin E) is a commonly used antioxidant, and an efficient supplement to prevent lipid oxidation in lipoproteins (Kagan et al., 1990). Research shows that ascorbate (vitamin C) acts similarly as a supplement and works well in combination with vitamin E (Bendich et al., 1986).

A 2005 study examined the effects of an antioxidant combination on oxidative stress levels and antioxidant defenses of trained horses (Moffarts et al., 2005). Over a twelve-week period, researchers gave forty Thoroughbreds in a 3-month training program a combination antioxidant supplement mainly consisting of ascorbic acid (vitamin C) (Twydil Racing Programme) (Moffarts et al., 2005). The supplement consisted of the following: ascorbic acid (11,500mg), alpha-tocopherol acetate (700 mg), beta-carotene (500 mg), copper (187 mg), zinc (769 mg), and selenium (7 mg) (Moffarts et al., 2005). In week 0, week 6, and week 12, workers obtained blood samples (Moffarts et al., 2005). The results indicated an increase in GPx concentrations from week 0 to week 12 for the group supplemented with the antioxidant combination (Moffarts et al., 2005). SOD

decreased over time in both the control and antioxidant groups (Moffarts et al., 2005). The control group and the experimental group were also different on week 12, where the antioxidant group had a higher GPx value (Moffarts et al., 2005). SOD concentrations did not show differences between the control and antioxidant group (Moffarts et al., 2005). SOD decreased in both groups by the end of the trial, but the antioxidant group had greater decline (Moffarts et al., 2005). The increase in GPx may be an indicator that this antioxidant supplement has the abilities to reduce damage caused by oxidative stress due to the increase in GPx which helps reduce the oxidative stress.

A 2016 study examined the effects of supplementing a combination of Vitamin E and polyunsaturated oil high in Omega-3 and Omega-6 fatty acids to mature horses (Mélo et al., 2016). Workers provided two groups of horses with different amounts of the supplement for eight weeks based on their activity levels. The maintenance group received 100 ml and the athletic group received 300 ml (Mélo et al., 2016). Unlike the earlier study described, there were no changes found in GPx activity with either group, however, there was a temporary increase indicated between the week before and four weeks after supplementation for both groups (Mélo et al., 2016). By the final week of supplementation, results showed a reverse of this increase in GPx for both groups (Mélo et al., 2016). From the results, the combination of Vitamin E and the oil supplement do not provide a long-lasting effect on the horses' reaction to oxidative stress in either type of activity levels.

Researchers conducted a study examining horses supplemented with a combination of Vitamin E and Vitamin C during an 80-km endurance race (Williams et al., 2004). Results indicated no effect on antioxidant status in either group but did find a

decrease in GPx in relation to distance raced (Williams et al., 2004). The Vitamin E and Vitamin C combination study results were similar to the study examining Vitamin E and polyunsaturated oil (Mélo et al., 2016).

These varying results found among the studies, maybe due to the varying amounts present in the supplements given to the animals. Researchers have found that Vitamin E and C influence oxidative stress due to their abilities to supply chemicals that combat that overabundance of ROS in the cell after exercise. An 80-km endurance race may not provide enough time for the supplement to cause an effect. The Vitamin E and oil combination may not have a high enough amount of the vitamin in the supplement to affect the body's oxidative levels in a beneficial manner.

MSM Methyl sulphonyl methane (MSM) is a sulfur-containing compound that has been found to have an indirect, positive effect on oxidative stress, and believed to play a role in the synthesis of glutathione (Parcell, 2002). Marañón and colleagues (2008) examined the antioxidant effects of MSM and a combination of MSM and Vitamin C. They found GPx activity was higher in the supplemented group than in the control throughout supplementation, but both declined compared to day 0 (Marañón et al., 2008). These results vary from the previous studies that all found elevated levels of GPx throughout supplementation, but it is similar to the results indicating higher levels of GPx in the supplemented group (Marañón et al., 2008).

Selenium A study examined the effects of selenium, an antioxidant supplement that is a co-factor for GPx, on oxidative stress in young equine athletes (Yavari et al., 2015; White

and Warren, 2017). Horses in the study received different concentrations of selenium in the diet and received different exercise regimens with varying levels of training (White and Warren, 2017). After 14 weeks of supplementation and training, results showed no differences in GPx activity (White and Warren, 2017). However, the GPx and SOD activities were greater in horses receiving training than in those that were not being trained (White and Warren, 2017). These results are similar to the 2005 results of Moffarts and colleagues, despite a different antioxidant supplementation. The combination administered by Moffarts also contained a small percentage of selenium (Moffarts et al., 2005).

RBO Another possible antioxidant used as a supplement for horses is gamma oryzanol, a naturally occurring antioxidant in rice bran oil (Juliano et al., 2005). Gamma oryzanol is a combination of ferulic acid esters of sterol and triterpene alcohols which is connected antioxidant activity on the stabilization of lipids (Wilson et al., 2007). Although research into the effects is limited, gamma oryzanol is an ergogenic aid given to dogs, horses, and humans (Ostaszewski et al., 2012). Rice bran oil also contains Vitamin E which has antioxidant effects. It has been suggested that the components act together to combat oxidative stress in the body (Huang, 2003). Limited research has been conducted towards the effects of supplementation of rice bran oil in the equine diet.

Muscle damage was examined by a study and its correlation to the supplementation of gamma oryzanol and beta-Hydroxy-beta-Methylbutyrate (Ostaszewski et al., 2012). Beta-Hydroxy-beta-Methylbutyrate is a metabolite of the

amino acid leucine and has been supplemented in order to reduce muscle wasting in the body (Ostaszewski et al., 2012). Both compounds were supplemented to young Thoroughbred racehorses that were involved in an intensive training program for 16 weeks (Ostaszewski et al., 2012). The experiment did not examine oxidative markers, and examined variables that analyzed muscle damage caused by exercise that is commonly in relation to oxidative stress (Ostaszewski et al., 2012). Gamma oryzanol alone did not influence muscle damage caused by exercise. However, when supplemented in combination with beta-Hydroxy-beta-Methylbutyrate, there was a decrease found in the variable associated with muscle damage (Ostaszewski et al., 2012). This research suggests gamma oryzanol is not a strong enough supplement to influence muscle damage, but used with compounds that have similar effects there may be a strong enough influence to decrease damage caused by exercise and oxidative stress.

Excluding the selenium supplement, the other studies that supplemented the antioxidants found that there was at least an increase in GPx activity. The increase in GPx results is an indicator that the antioxidant supplements help prevent damage caused by oxidative stress during bouts of exercise. The positive results are a possible indicator that these supplements may have abilities to impact muscle damage caused by oxidative stress. The selenium study seemed to have an issue of not involving a control group for the study as well as the study examining Vitamin E supplement which may decrease the value of the results that they provided (White and Warren, 2017; Mélo et al., 2016).

Conclusion

Antioxidant supplementation has shown positive effects on the body in that it can help reduce damages caused by oxidative stress. The research presented has provided indicators that an increase in the number of antioxidants in the diet can possibly improve the population of enzymes that help regulate the effects of oxidative stress. Through a thorough examination of oil supplementation for its lipid accessibility, researchers have found an increase in variables that help transport these fats and suggest increased utilization.. From the few studies examining rice bran oil supplementation in the equine diet, past research has indicated that this oil can act as an efficient fat supplement (Frank et al., 2004). Even though previous research has not looked specifically at oxidative stress and antioxidant markers, the decrease in muscle damage may be correlated to the reduction of oxidative stress that may be causing this damage (Ostaszewski et al., 2012). However, the reduction was only correlated when gamma oryzanol was supplemented with another compound that can relate to antioxidant abilities. Due to lack of sufficient research, the antioxidant abilities of rice bran oil alone in the equine diet is still relatively unknown.

As rice bran oil has the possibility of acting as both a fat and antioxidant supplement in the equine diet due to the presence of gamma oryzanol and high number of Omega-6 fatty acids in the oil itself, it may prove to be a useful dietary supplement. Still, the presence of gamma oryzanol and the possible anabolic effects it may have has left the question of whether the supplement can be used in high performance horse competitions. In 2009, the Federation Equestre Internationale (FEI) temporarily banned the

supplementation of gamma oryzanol due to its possible effects on performance (Mösseler et al., 2010). However, little research has actually examined and proved these effects occur in the horse. To better understand the use of rice bran oil as a supplement, research is needed to examine variables related to fat carriers such as NEFAs or triglycerides as well as oxidative stress variables such as GPx and TAS. This research will help further confirm or deny the antioxidant and fat supplement effects that rice bran oil may have on the horse.

CHAPTER TWO: A COMPARISON OF RICE BRAN OIL AND CORN OIL IN THE EQUINE DIET

Introduction

In the equine diet, fat supplementation is commonly practiced in order to increase caloric density or improve performance. Fat supplements provide another source of energy for the horse in addition to forages and grain. Oil supplementation provides a highly digestible source of lipids which play an important role in many metabolic pathways in the body (Crandell et al., 1999). Lipids are used as a source of energy during skeletal muscle metabolism and provide a densely packed form of energy when these muscles are in use (Rose et al., 1980). Fats also provide an additional source of essential fatty acids known as Omega-6 and Omega-3 which are not naturally produced in the horse's body and must therefore be provided in the diet, either through fat supplementation, forages, or grain (O'Connor et al., 2004).

Some dietary supplements have also been examined to determine their effects on the horse's response to oxidative stress. Oxidative stress is the imbalance between free radicals and antioxidants present in the body. Free radicals, or reactive oxygen species (ROS), are oxygen-containing molecules that are not reduced completely in the blood (Williams, 2016). These molecules can damage DNA, lipids, and proteins if not regulated correctly (Williams, 2016). Research involving rats, humans, and horses showed this connection to muscle damage in these species (Powers and Jackson, 2008). Providing an increase in antioxidants to their already existing supply could help

balance the accumulation of ROS in the system and reduce the risk of damage to muscle tissue.

Rice bran oil (RBO) has gained attention as a fat source for its palatability, economic affordability, and potential antioxidant effects, as well as its suggested anabolic effects. Limited research has been done to evaluate the effects of RBO when supplemented in the equine diet. Rice bran oil naturally contains gamma oryzanol, a combination of ferulic acid esters of sterol and triterpene alcohols which is connected to antioxidant activity on the stabilization of lipids (Wilson et al., 2007). Although research is limited, gamma oryzanol has been found to work as an ergogenic aid given to dogs, horses, and humans (Ostaszewski et al., 2012).

The objective of this current study is to examine the effects of rice bran oil, as compared to corn oil, as a fat supplement in the diet of mature horses. The study also examines the effects of rice bran oil on antioxidant populations and on oxidative stress.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee of Middle Tennessee State University (Protocol 18-2016).

Horses

Twelve healthy, mature (16.8 ± 8.8 years) horses of various breeds were obtained from the teaching and research herd of MTSU Horse Science. The four mares and eight geldings were randomly assigned to two groups of six, balanced by sex. Prior to the study, the horses were used in teaching lessons, with week-day stalling and pasture

access each weekend. Once the study began, horses were continuously stalled (9.3m2) for two separate five-week periods, with a three-week washout between periods, during which pasture access was again permitted. Throughout the study, all horses received a commercial pelleted concentrate (Purina Strategy) and a prairie grass hay to meet or exceed NRC requirements for light work. Horses were randomly assigned to either corn oil (CO; Gordon Food Service Store, Murfreesboro, TN) or rice brain oil (RBO; Riceland Foods, Stuttgart, AR) for the first treatment period. They then received the other oil during the second period. Oil was top-dressed onto the concentrate portion of the ration at a rate of 324 mg/kg BW/d. Horses were also provided ad libitum access to water. Throughout the study, horses continued to be lightly exercised at least three times per week for 30-45 minutes, either in lessons or on a mechanical panel exerciser.

Sample collection

Prior to the start of the study (Day 0), at the midpoint of each treatment (Day 17), and at the conclusion of treatment (Day 35), body weight and morphometric measurements were taken and blood was collected for later analysis. Specifically, venous blood was collected via jugular venipuncture into 10 ml glass serum and plasma (EDTA) tubes. Samples were centrifuged at 3000 rpm for 15 minutes, and were then aliquoted to labeled micro tubes before being stored at -80C.

Non-esterified Fatty Acids and Triglycerides

Blood was then placed in a freezer and later analyzed for non-esterified fatty acids (NEFA) and triglyceride (TG) concentrations using Wako Diagnostics for instructions and laboratory kit supplies (Wako Diagnostics, Richmond, VA).

TG concentrations were measured using the enzymatic method L-Type Triglyceride M which includes N-(3-sulfopropyl)-3-methoxy-5-methylaniline which causes a blue pigment to form in the presence of triglycerides (Wako Diagnostics, Richmond, VA). The process isolates triglycerides from other forms of lipids, and the concentrations are then obtained through measuring absorbance using a microplate reader at wavelengths between 600 to 700 nm (Bio-Rad Laboratories, Hercules, CA).

NEFA concentrations were measured using a similar enzymatic method through the acylation of coenzyme A caused by the fatty acids present in the sample (Wako Diagnostics, Richmond, VA). Through numerous chemical reactions the samples in combination with other chemicals form a reaction where a purple coloration is formed. The absorbance of this coloration is then measured at a wavelength 550nm.

Total Antioxidant Status

Total antioxidant status (TAS) was analyzed through plasma samples obtained from the blood samples collected. TAS concentrations were measured using Cayman's Antioxidant Assay (Cayman Chemical, Ann Arbor, MI). TAS concentrations were evaluated by relying on the ability of antioxidants to inhibit the oxidation of 2,2'-Azino-di-[3-ethylbenzthizoline sulphonate] which is provided in the Cayman kit. The absorption of the sample prepared using this assay kit is measured at a wavelength of 750 nm or 405 nm using the microplate reader referenced earlier. Absorbance concentrations are then recorded and analyzed as total antioxidant status.

Rump Fat Thickness

Rump fat thickness was measured using an ExaGo ultra sound monitor (ECM, France) that measured the space between the muscle and skin where subcutaneous fat is

easily identifiable. The measurement was taken from the middle gluteal area 10 cm caudal to the sacrum and 10 cm from the midline. The area was measured on day 0 and then shaved to maintain consistency. Before placing the probe onto the area, the skin was again shaved and sprayed with alcohol or lathered in a lubricating gel.

Gaskin and Forearm Circumference

Gaskin and forearm circumference were measured with a soft measuring tape, at the mid-point of both the gaskin and forearm. Again, the area was shaved to maintain consistency.

Body Weight

Body weight was obtained using a digital scale (Transcell TI-500 Industrial Scale, Trancell Technology, INC, Buffalo Grove, IL). All measurements were initiated at approximately 0900h on each collection day.

Statistical Analysis

Data were analyzed using a mixed model ANOVA with repeated measures in SAS 9.2 (SAS, Cary, NC). Effect of treatment, day, and treatment*day were evaluated. Significance was defined as $P < 0.05$ and trends were considered at $P < 0.10$. Data are presented as mean +/- SEM.

Results

Body Weight

Body weight was not different due to treatment ($P = 0.12$) or day*treatment ($P = 0.92$). Body weight demonstrated a decrease over time ($P < 0.05$), being lower on Day 35 (533 ± 16.9 Kg), than Day 17 (546 ± 16.9 Kg), or Day 0 (557 ± 16.9 Kg).

Rump Fat Thickness

Rump fat thickness was not different over time (P = 0.06), due to treatment (P = 0.68), and day*treatment (P = 0.95). There was a trend indicated by day, and specifically Day 0 to Day 17 showing a decrease (P = 0.0505). The overall mean of rump fat thickness did show a slight decrease over time, from Day 0 (11.1247 ± 1.3 mm) to Day 35 (10.8282 ± 1.3 mm), when examining the mean of all horses (Figure 1).

Gaskin Circumference

Gaskin circumference decreased over time (P < 0.05), being lower on Day 35 (46 ± 0.7 cm) than Day 0 (47 ± 0.7 cm, Figure 2). Gaskin circumference was not different due to treatment (P=0.70) or interaction (P = 0.22).

Forearm Circumference

Forearm circumference decreased over time (P < 0.05), but was not different due to treatment (P = 0.34) or interaction (P = 0.78). Forearm circumference decreased over time Day 0 (47 ± 0.8 cm) to Day 35 (45 ± 0.8.cm, Figure 3).

NEFA

Non-esterified fatty acid concentrations decreased over time (P < 0.05, Figure 4), being lower on Day 35 (94 ± 14.2), than Day 17 (98 ± 14.2), or Day 0 (142 ± 14.4). Concentrations were not different due to treatment (P = 0.46) or interaction (P = 0.65)..

Triglycerides

There was no changes or differences found in relation to the triglyceride concentrations over time (P = 0.43), due to treatment (P = 0.70), or interaction (P = 0.92).

TAS

There was no changes or differences found in relation to the total antioxidant status over time (P=0.84), due to treatment (P=0.16), or interaction (P=0.75).

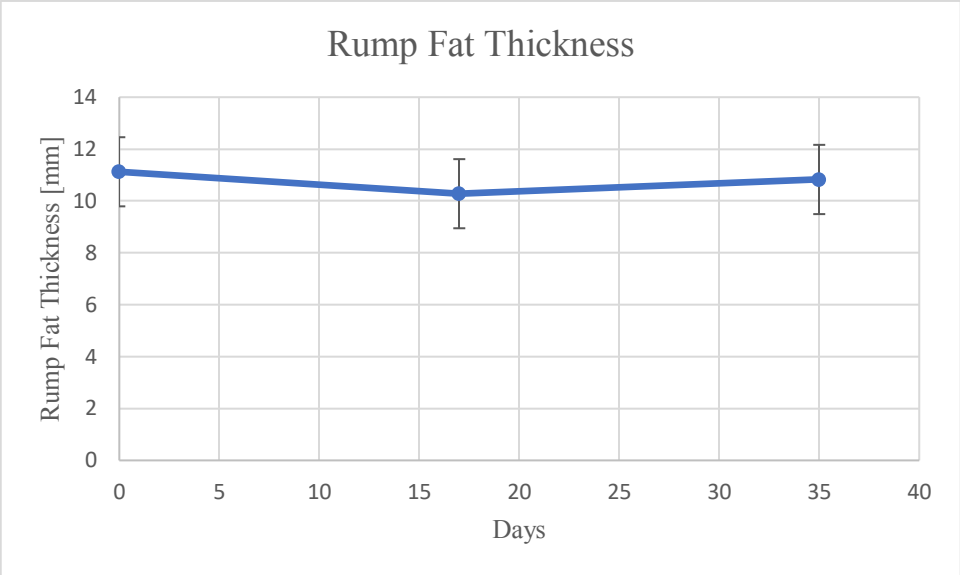

Figure 1. Rump fat thickness of horses fed either RBO or CO measured on Day 0, Day 17, and Day 35. A trending decrease over time (P = 0.0505).

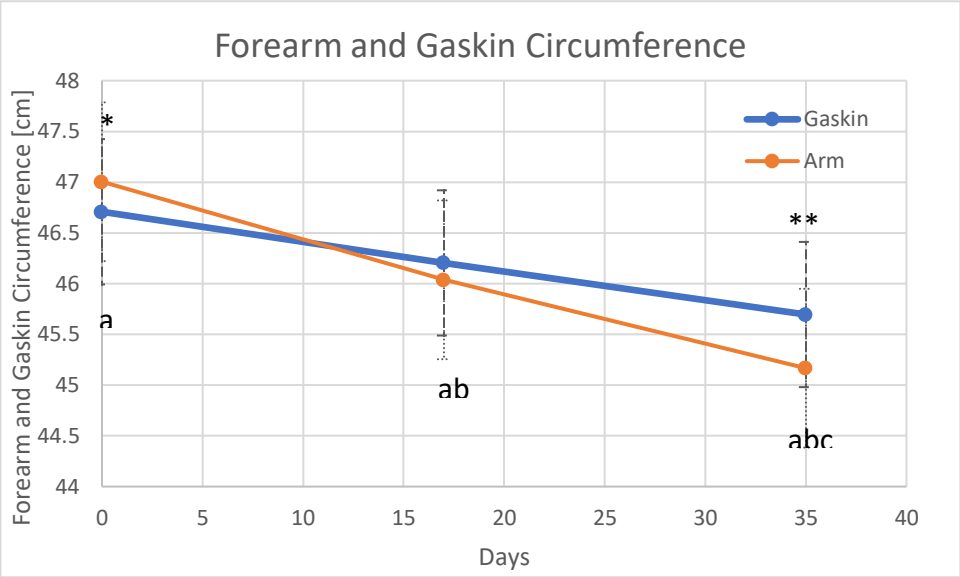

Figure 2. Forearm and Gaskin Circumferences of horses fed either RBO or CO on Day 0, Day 17, and Day 35. Both indicate a decrease over time ($P < 0.0001$ and $P = 0.0017$, respectively). Letters for forearm circumference indicate ($P < 0.05$). * for gaskin circumference indicate ($P < 0.05$).

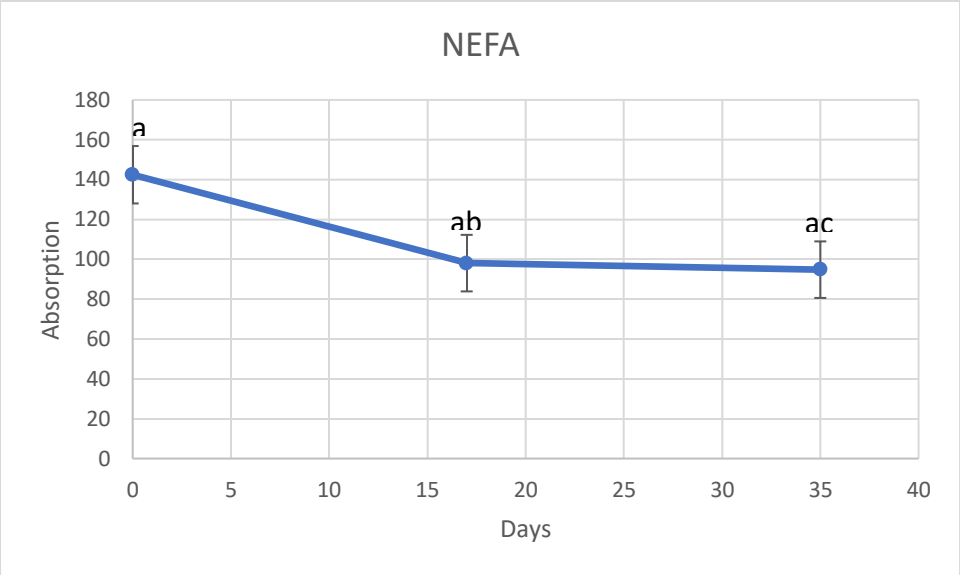

Figure 3. NEFA concentrations of horses fed either RBO or CO on Day 0, Day 17, and Day 35. Concentrations decreased over time ($P = 0.0065$). Letters indicate ($P < 0.05$).

Discussion

Fat supplementation is commonly used in the equine diet in order to improve caloric density or performance. Fish oil and corn oil have been heavily evaluated as supplements due to their Omega-3 and Omega-6 fatty acid concentrations and the effects they have on the body. Rice bran oil is another fat supplement that can be used in the equine diet and has been found to have high population of Omega-6 fatty acids. The oil also contains gamma oryzanol which acts as an antioxidant and may have ergogenic effects when supplemented in the equine diet (Juliano et al., 2005). At this point in time, very little research has examined the effects of rice bran oil as a possible fat or antioxidant supplement in the equine diet. The objective of this study is to compare the effects of rice bran oil to those of corn oil over a 35-day period. This study indicates that rice bran oil had a similar effect on the horse as corn oil when supplemented in this study. No differences were found between the two oils which indicates that rice bran oil can serve as a suitable fat supplement in the equine similarly to corn oil. In most of the variables evaluated there was a decrease, specifically in body weight, gaskin and forearm circumference, and NEFA concentrations.

NEFA and triglyceride concentrations can indicate how the fatty acids are being utilized in the body when consumed. NEFAs themselves are used as an indicator of the ratio between fatty acid mobilization from adipose tissue and fatty acid utilization in muscular tissue (Monteverde et al., 2017). Triglycerides serve as a source of fatty acids during muscle metabolism (Monteverde et al., 2017). In this current study, there was a decrease found in NEFA concentrations over time when examining both oils. There were no differences found between the oils themselves. An older study examined rice bran oil,

found a decrease in NEFA concentrations after five weeks of supplementation (Frank et al., 2005). A decrease in triglycerides was also indicated by the study after rice bran oil supplementation (Frank et al., 2005). Another study found no changes in triglyceride levels after supplementation of corn oil for 63 days (Connor et al., 2004). A decrease in NEFA concentrations can indicate that both rice bran oil and corn oil can be fully utilized in the body once provided in the equine diet. The contradiction of triglyceride results between the current study and the study conducted by Frank (2004) indicates that further research is needed in order to better understand how RBO can affect triglyceride concentrations in the horse.

The gaskin and forearm of the horse are areas where there are large amounts of muscling and were used as an indicator of whether the oils had a conformational effect. Body weight was also a variable used to determine if there was a change in conformation caused by the oils themselves. All variables decreased overtime for both oil supplements. Frank (2005) indicated an increase in body weight when examining rice bran oil, and this can correlate to the increase in fat supplemented in the diet. However, there was a decrease in body weight in this study. The decrease can be explained by the horses not being conditioned to the amount of exercise they were exposed to once supplementation began. The horses were turned out on pasture with no forced exercise for a three-month period during the summer prior to supplementation. When observations began, the horses were put into lessons and exercised at least three times a week for 30 minutes at a time. The introduction of exercise caused the horses to become more fit which led to weight loss and a decrease in conformational areas listed.

Examining rump fat thickness throughout the study allowed for a better understanding of whether the fat supplement was being utilized in the body or being stored in adipose tissue. There was a trend of rump fat thickness decreasing over time for both oil supplements. There were no differences between the oils themselves. This could again be due to the horses becoming more conditioned to the exercise they were introduced to during the study.

Oxidative stress is the imbalance between free radicals and antioxidants present in the body. Free radicals, or reactive oxygen species (ROS), are oxygen-containing molecules that are not reduced completely in the blood (Williams, 2016). These molecules can damage DNA, lipids, and proteins if not regulated correctly (Williams, 2016). The body can naturally protect itself from ROS accumulation by producing enzymes that help maintain a balance if there is a high level of oxidants (Kuhn, 2003). These enzymes, known as antioxidants, naturally react to ROS which neutralizes them. Antioxidants can also be supplemented in the diet in order to help prevent damage caused by oxidative stress by combating the high number of free radicals in the blood. Total antioxidant status (TAS) can be measured in order to gauge how a supplement affects oxidative stress by measuring the capacity of antioxidants in the blood (Lykkesfeldt andSvendsen, 2007). TAS was measured to see if rice bran oil contributed to antioxidant populations in the body. Corn oil has been a heavily researched oil and is known to only act as a fat supplement in the diet. When examining the results, there were no differences found between the two oils. There were also no changes indicated overtime. Earlier research has indicated that gamma oryzanol can decrease muscle damage caused by oxidative stress in horses when supplemented with beta-

hydroxy-beta-methylbutyrate which has also been indicated to decrease muscle damage as well (Ostaszewski et al., 2012). The current study did not indicate that RBO has an effect on oxidative stress. However, limited research has been done to examine rice bran oil as an antioxidant supplement in the equine diet. It may be more beneficial to have horses in an intensive exercise program instead of light work to induce oxidative stress in the body, and examine the effect of RBO supplementation.

Due to the change in exercise, the decrease in gaskin and forearm circumference and body weight can be expected when the horses were adjusting to exercise. There were no observations made from this study that may indicate rice bran oil is a more beneficial supplement than corn oil, but it has the potential of providing the same effects. The results of this study indicate that rice bran oil does not have an effect on antioxidant populations when supplemented in the diet. It does confirm that it can be safely administered in the equine diet and was equally as palatable as corn oil. Rice bran oil is a safe fat supplement to give in the equine diet based off of this current study.

Conclusions

It can be concluded from this study that rice bran oil can have similar effects to corn oil when supplemented into the equine diet. It is difficult to say that it increased caloric density in the diet due to the decrease in body weight and rump fat thickness for both oil groups, though it is likely that this is due to the increase in the horses' workload. A decrease in NEFA concentrations, and no change in TG concentrations can indicate a lack of an effect of the fat supplement for both groups. The results may have been more conclusive if they had already been conditioned to the current workload, but more research is needed to say for certain. However, the oil was palatable and had the same

results as corn oil, which is a well-researched supplement known for its caloric density, which suggests that this can be a beneficial supplement in the diet. More research is needed for more accurate results that included horses already acclimated to any workload they are involved in.

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APPENDICES

APPENDIX A: IACUC APPROVAL

IACUC
INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

IACUCN001: PROTOCOL APPROVAL NOTICE

Thursday, July 19, 2018

Senior Investigator	**Holly Spooner** (ROLE: Principal Investigator)
Co-Investigators	John Haffner and Morgan Garrick
Investigator Email(s)	*holly.spooner@mtsu.edu; john.haffner@mtsu.edu; mwg2v@mtmail.mtsu.edu*
Department	Horse Science Center
Protocol Title	***Oxidative stress in the horse: Influence of corn vs. rice bran oil & influence of infrared emitting fabric technology***
Protocol ID	**18-2016**

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the ***Designated Member Review (DMR) mechanism*** and has approved your protocol in accordance with PHS policy. A summary of the IACUC action(s) and other particulars of this this protocol is tabulated as below:

IACUC Action	**APPROVED for one year from the date of this notification**	
Date of Expiration	**7/31/2019**	
Number of Animals	18 (EIGHTEEN)	
Approved Species	**MTSU Teaching Herd**	
Category Subclassifications	☐ **Teaching** ☐ Classroom ☐ Laboratory	☐ **Research** ☐ Laboratory ☐ Field Research ☐ Field Study ☐ Handling/Manipulation ☐ Observation
	Comment: NONE	
Approved Site(s)	MTSU Horse Science Barn	
Restrictions	**Satisfy DMR requirements AND annual continuing review**	
Comments	NONE	
Amendments		NONE

This approval is effective for three (3) years from the date of this notice. This protocol **expires on 7/31/2021** The investigator(s) MUST file a Progress Report annually regarding the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be approved by the IACUC prior to

7/31/2019 for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule: Refer tp the following table to request your CR:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	6/30/2019	TO BE COMPLETED
Second year report	6/30/2020	TO BE COMPLETED
Final report	6/30/2021	TO BE COMPLETED

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

Post-approval Protocol Amendments:

Date	***Amendment(s)***	***IRB Comments***
NONE	NONE	NONE

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. Be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

Compliance Office
(On behalf of IACUC)
Middle Tennessee State University
Tel: 615 494 8918
Email: iacuc_information@mtsu.edu (for questions) and
iacuc_submissions@mtsu.edu (for sending documents)