EFFECTS OF CRUDE RICE BRAN OIL AND A FLAXSEED OIL BLEND IN YOUNG HORSES ENGAGED IN A TRAINING PROGRAM

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DEDICATION

To my dad, who I know would be proud to see me doing what I love, following my dreams, and making the most of every opportunity.

ABSTRACT

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Rice bran oil and a flaxseed oil contain omega-3 fatty acids with the potential to reduce post-exercise inflammation and muscle damage. This study observed body fat and muscle parameters, heart rates, and plasma lactate, glucose, interleukin-1β, creatine kinase, and fatty acid profiles in lightly worked young horses undergoing a 16 min incremental exercise test (IET; 16.1, 19.3, 22.5, and 25.7 kph) after 60-d of oil inclusion. Horses received their energy requirement as 40% from concentrate and 60% from hay, with oil replacing 25% of concentrate calories. Treatments consisted of CON (n=4), which received no oil, FLAX (n=4), which received a flaxseed oil blend, and RICE (n=4), which received crude rice bran oil. Blood was obtained pre-exercise, 1 min, 30 min, 24 h, 48 h, and 72 h post-IET. Data were analyzed by repeated measures ANOVA. All treatments increased ($P < 0.05$) in body fat and muscle parameters after 30-d of the study. Plasma lactate and glucose concentrations were greater ($P < 0.05$) 1 min post exercise in all treatment groups, and all treatment groups exhibited heart rates greater than 150 beats per min (bpm), indicating anaerobic exercise. Plasma creatine kinase activity was not different in CON during the study, greater ($P < 0.05$) in RICE from pre-exercise to 30 min post exercise overall, and lesser ($P < 0.05$) in FLAX at 30 min post exercise on day 30 compared to day 0. Plasma interleukin-1β was greater ($P < 0.01$) in CON on day 60, but no differences were shown in FLAX and RICE throughout the study. Plasma alpha-linolenic and linoleic acids were highest ($P < 0.05$) in FLAX after 30-d of inclusion, while CON horses had greater ($P < 0.05$) EPA overall and DHA after 60-d.

These results indicate that 30 to 60-d of inclusion of crude rice bran oil or a flaxseed oil blend may benefit lightly worked young horses by reducing training program related increases in interleukin-1β, while only the flaxseed oil blend reduced exercise induced increases in creatine kinase. Results also indicate that neither oil induces loss of muscle mass nor increase in body fat. Additionally, the flaxseed oil blend has the potential to increase plasma omega-3 and omega-6 fatty acids.
KEY WORDS: Equine; Flaxseed oil; Rice bran oil; Inflammation; Muscle damage

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PREFACE

“If we knew what it was we were doing, it would not be called research, would it?”

– Albert Einstein

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CHAPTER I

Introduction

Horses participate in a variety of sports, all of which have various levels of exercise induced stress. Some of the high intensity, short duration sports may include Thoroughbred racing, barrel racing, calf roping, and draft horse pulling contests. Alternately, low intensity, long duration sports may include endurance racing, ranch horse work, and competitive trail rides. There are other sports that may not fit exclusively into either of these categories, such as show jumping, which can combine both high and moderate intensity work. A commonality between all these endeavors is that horses may suffer from inflammation and muscle pain due to muscular damage, which could inhibit their ability to continue competing or working. Evaluating the severity of this inflammation can be determined by observing markers of metabolism, muscle breakdown, and inflammation, such as lactate, glucose, interleukin-1β (IL-1β), and creatine kinase (CK) in plasma. Determining concentrations and activity of these biomarkers in plasma allows for their use as an indicator of inflammatory processes because systemic markers indicate total inflammatory activity (McIlwraith, 2005; Sumer et al., 2006). A variety of nutritional supplements and dietary modifications have been attempted in order to reduce this inflammation and muscle damage associated with exercise.

In the equine industry, both rice bran oil and flaxseed oil have long been used as multipurpose equine nutritional supplements. Both oils contain components that reduce oxidative damage and inflammation including phytosterols, and vitamin E which contains tocopherols and tocotrienol (Sembratowicz et al., 2020). Both oils contain essential fatty

acids; however, flaxseed oil contains higher amounts of the anti-inflammatory omega-3 fatty acid, alpha-linolenic acid (ALA), than does rice bran oil. Generally, rice bran oil has a higher omega-6 to omega-3 ratio (19:1), flaxseed oil is lower, at 1:3 (Cleland et al., 2006). While there is no ideal ratio, it has been suggested that providing a diet with a higher omega-3 to 6 ratio can be more beneficial by helping to reduce inflammation and muscle damage post exercise. Additionally, rice bran oil contains γ-oryzanol, a mixture of antioxidant compounds that have previously been shown to lower blood lipids and oxidative stress (Juliano et al., 2005; Bumrungpert et al., 2019). In general, dietary fat supplementation increases caloric density, as well as improves thermoregulation and reduces lactic acid concentrations during intense exercise in horses. Adapting horses to a high fat diet has also shown to increase fatty acid oxidation, which in turn spares utilization of muscle glycogen and blood glucose (Kronfeld, 1996; Kronfeld 1994; Swirsley et al., 2017). Further, horse owners use fat supplements for improved coat condition and the above-mentioned nutritional benefits (Swirsley et al., 2017).

The objective of this study was to determine the effects of replacing 25% of concentrate calories with either crude rice bran oil or a flaxseed oil blend on plasma concentrations of lactate, glucose, IL-1β, CK, and fatty acids, heart rates after intense exercise, body fat estimates, and muscle scores in young horses engaged in an introductory ground training program.

We hypothesized that horses consuming either oil would have reduced plasma lactate, and IL-1β concentrations, reduced plasma CK activity, and increased plasma omega-3 fatty acids. We also hypothesized that replacing concentrate calories would not result in muscle mass loss or body fat gain.

CHAPTER II

Literature Review

Body Weight, Body Fat, and Exercise Capacity

In human athletes, it has been accepted that nutritional status can influence exercise capacity and success, even in people that are not overweight (Lewis et al., 1986). In equine athletes, determining this nutritional status can be done by assessing multiple variables. This includes fat parameters such as body weight (BW), body condition score (BCS), cresty neck score (CNS), intermuscular fat (IMF), and rump fat thickness (RFT), as well as muscle parameters such as topline evaluation score (TES), forearm and gaskin circumference, and rib eye area (REA). In horses, field studies have been conducted to investigate the relationship between body condition, body composition, and exercise performance in endurance horses (Garlinghouse and Burrill, 1999; Lawrence et al., 1992), Standardbred trotters (Kearns et al., 2002; Leleu and Cotrel, 2006), and Thoroughbred racehorses (Fonseca et al., 2013). These studies indicate that BCS and BW play an integral role in endurance horses and their ability to complete races, as well as percent body fat affecting performance in Standardbred trotters and Thoroughbred racehorses.

Body Weight

The average BW of an adult stock type horse is typically between 462 and 568 kg (Martinson et al, 2014), with 2-yr old horses being at about 90% of their mature BW (NRC, 2007). Body weight in horses can be measured using a livestock scale, weight tape, or estimation formula of Wagner and Tyler (2011):

$$\text{Body weight} = (\text{heartgirth}^2 \text{ (in)} \times \text{body length (in)}) / (11{,}880 \text{ cm}^3)$$

Body weight can be greater or lesser than average depending on body fat, measured using BCS, CNS, IMF and RFT. Muscling also contributes to body weight, which can be evaluated using TES, forearm and gaskin circumference, and REA.

Body Condition Score

Body condition score (BCS) is a subjective estimation of subcutaneous fat cover in horses and other livestock species. Six different areas of the horse including the neck, withers, shoulders, loin, tailhead, and ribs are physically palpated and assigned a score on a scale of 1 (poor) to 9 (extremely fat). This system was designed by Henneke et al. (1983) and is one of the most widely used methods of body condition scoring. Even though this method was developed for Quarter Horse mares, it has been used on horses of various breeds, ages, and sex. Since it has not been determined if the class of the horse, location of the fat, or type of diet influences fat accumulation, this may compromise the system's ability to judge other breeds of horses. In a study done by Suagee et al. (2008), BCS was evaluated in mature Thoroughbred geldings to see if scores were affected by diet and weight gain. In another study by Suagee et al. (2013), a mix of light breed horses of all sexes, ranging in age from 4 to 20 years, were evaluated to determine BCS. Jensen et al. (2016), also used the same system to evaluate BCS in Icelandic horses ranging in age from 4 to 26 years, and included geldings, mares, and stallions. These previous studies indicate that BCS could be used as a representation of the energy reserves in various breeds, ages, and sexes of horses.

Cresty Neck Score

Cresty neck score is measured with a visual and tactile assessment of the nuchal crest of the neck, with a number between 0 and 5 being assigned (Giles et al., 2015). This

system designed by Carter et al. (2009), states that a score of 0 indicates no palpable crest, a score of 1 indicates no visual appearance of a crest but slight filling can be felt, a score of 2 indicates a noticeable appearance of a crest with fat deposited evenly from poll to withers, a score of 3 indicates a large and thickened crest with heavier fat deposits in the middle of the neck, a score of 4 indicates a grossly enlarged and thickened crest that can no longer be cupped in one hand, and a score of 5 indicates a large crest that permanently droops to one side. Evaluating CNS is subjective and is a common method for determining nuchal crest adiposity in horses. An increase in fat deposits along the crest has been associated with a greater incidence of developing certain metabolic disorders such as insulin resistance and laminitis (Treiber et al., 2006).

Topline Evaluation Score

Muscle development in the back, loin, and croup of horses can be evaluated using topline evaluation scoring (TES) created by Progressive Nutrition (Hopkins, MN). These three areas are easy to identify as well as a good way to indicate the muscle status in horses. This system is both visual and tactile, as well as subjective, with scores being assigned the letter A, B, C, or D based on topline status. The letter A indicates that the horse has ideal muscle development throughout the entire topline. The letter B indicates signs of concave withers as well as the back between the vertebrae and the top of the ribs. The letter C indicates all signs shown with a B score, along with signs of concave loin areas. The letter D indicates a concave topline in all three areas, with signs of muscle atrophy, and visible backbone and hip bones (Progressive Nutrition, Hopkins, MN). However, there have been no directed studies to confirm this TES system, or to support that these measurements can be used to indicate back strength or muscling (Smith, 2016).

Forearm and Gaskin Circumference

Forearm circumference and gaskin circumference are commonly recorded to monitor muscle accretion and are the easiest measurements to indicate muscling in an animal. A study done by Cunningham and Fowler (1961), observed forearm circumference in Quarter Horse stallions and mares, with measurements taken at birth, and again at 3, 6, 12, 18, 24, 36, 48, and 60 months of age. Results showed that females reached forearm muscle maturity at 18 months of age, while males did not fully mature until almost 60 months of age. The correlation between age and forearm circumference was low ($r^2 = 0.51$) indicating that there are other factors besides age, such as physical activity, that play a critical role in developing the forearm muscle.

Rump Fat Thickness

Obtaining fat cover measurements via ultrasound is frequently used in equine nutrition research. This method originated from research conducted by Westervelt et al. (1976) on horses and ponies evaluated on fat thickness at the shoulder, rib, and rump. Rump measurements are taken 5 cm lateral from the midline at the center of the pelvic bone. Total body fat is then calculated using the equation $Y = 8.64 + 4.70X$; with X being rump fat thickness in centimeters and Y being percent body fat. This percentage was shown to correlate well with BCS ($r^2 = 0.65$) (Henneke, 1981). Multiple studies have identified a body fat percentage range using a variety of breeds. Ranges stated in Standardbreds by Kearns et al. (2002), Arabians by Lawrence et al. (1992), Thoroughbreds by Vick et al. (2006), and various breeds of mares by Powell et al. (2002), suggested that horses with an average BCS of 4 to 5, would have 7 to 11% body

fat. Horses with a 5 to 7 BCS average were said to have 12 to 24% body fat (Henneke, 1981; Kubiak et al., 1991).

Relationship Between BCS, BW, and Body Fat Percentage

Many studies have correlated percent body fat to BCS, as well as a gain in BW being needed to increase one BCS. Mature light breed horses have shown an average daily gain of 1 kg when fed an average of 2.8% of their BW. Body condition score increased from a 4 to a 6, with each one increase in score correlating to a 16 to 20 kg increase in BW (Heusner, 1993). A study done by Graham-Thiers et al. (1999) indicated that an average daily gain of 0.3 kg was achieved when horses received a daily intake of 7 Mcal above the average horse DE requirement; therefore, providing 23 Mcal DE daily is suggested to produce a 1 kg increase in BW per day in mature light breed horses.

Relationship Between Body Fat Percent and Exercise Response

In horses, studies have shown a relationship between body fat percentage and response to exercise. In a study conducted by Garlinghouse and Burrill (1999), all horses that had a BCS less than 3 were unable to complete a 160 km endurance race, while horses with a BCS of 3 had a completion rate of 9.5%. The overall completion rate for the race was 67%. Horses with a BCS of 5 and 5.5 had the highest completion rate at 90.7% and 100% respectively. Regression analysis showed that for each numerical increase of body condition score up to a 5.5, the horses completed an additional 31.81 km. Lawrence et al. (1992) concluded that the percent body weight for Arabian or part Arabian endurance horses that completed a 150-mile endurance race was about 6.5% or about 26 kg of fat, while the horses that could not finish had an average body fat percentage of 11% or approximately 45 kg of fat. In Standardbred horses, as well as

Thoroughbred racehorses, having a larger fat free mass was shown to improve race time and performance, and produced more positive results than horses who had a low-fat mass (Kearns et al., 2002; Fonseca et al., 2013).

Metabolism During Exercise

Evaluating athletic performance through analysis of lactate and glucose is becoming an essential part of managing training programs in horses (Ferraz et al., 2008). During an incremental exercise test (IET), blood lactate typically remains constant or slowly increases as the workload increases until the anaerobic threshold (4mm/l), at which point lactate concentrations rise quickly. This increase is driven by the fact that the rate of clearance of lactate from blood is surpassed by the rate of production in muscle (Kline, 1997). Lactate production and accumulation contributes to muscle fatigue by lowering the pH within cells, which then inhibits the contraction process (Kline, 1997). Kinderman et al. (1979) defended a model in response to lactic acid threshold values. This model stated that 2 mmol/l was the upper limit of aerobic metabolism, and therefore was referred to as the aerobic threshold. An aerobic-anaerobic transition occurs between plasma lactic acid concentrations of 2 and 4 mm/l. When plasma lactic acid levels increased to 4 mmol/l, this was considered to be the anaerobic threshold, a value at which lactate production surpasses removal, and successive performance becomes limited. It is important to note that plasma lactate concentrations are approximately 1.5 times greater than blood lactate concentrations (Pösö, 2002; Rainger et al., 1995).

Aerobic and anaerobic exercise can also be determined by looking at heart rates (HR). A bpm of 150-170 indicate that the anaerobic threshold has been met, while a

number below this range indicates aerobic exercise. Max HR in a mature horse appears to be between 220 and 260 bpm, while resting is 30-40 bpm (Freeman et al., 2003).

Studies have shown a relationship between glucose and lactate concentrations in an IET, therefore allowing plasma glucose to also be used for training evaluation in horses (Ferraz et al., 2008). It has been suggested that the glucose threshold in horses, 160 to180 mg/dl or 8.8 to 10 mmol/L, is defined as the exercise intensity in which glucose concentrations rise as the result of a hepatic release/uptake ratio increase (Simões et al., 2003; Kaneko et al., 2008). It has been shown that the release of adrenalin can promote rapid and potent control of glycogenolysis during exercise, which in horses is related to stress intensity (Nagata et al., 1999). Human studies also suggest a stress threshold where adrenergic activation occurs, and where adrenalin production stimulates glycogenolysis as well as lactate production (Simões et al., 2003). Glycogenolysis has been shown to occur for up to 4 h post exercise, with partial muscle glycogen repletion occurring within 24 h of exercise (Hyyppä et al, 1997); Davie et al., 1994, 1995; El Snow and Harris, 1991). Studies have shown that high fat diets can either improve performance or produce no change in glycogen repletion (Oldham et al., 1989; Eaton et al., 1995; Topliff et al, 1983). When improvement was observed, it was thought to be from increasing glycogen stores after fat supplementation, or from creating a glycogen sparing effect by increasing fat utilization during exercise. These varied results may be due to a difference in fat type and amount, treatment duration in relation to metabolic adaptations, or a washout period being utilized (Orme et al., 1997).

Role of Fat Adaptation to Reduce Lactic Acid Production

Adapting horses to a higher fat diet can lessen the amount of lactate that is produced during exercise by reducing glucose utilization. This is because muscle cells build more pathways for using fats, and fats themselves inhibit the use of glucose (Randle et al., 1963). When fatty acids are utilized for fuel, citrate and acetyl CoA, two chemicals in the Krebs cycle, increase causing phosphofructokinase (PFK) and pyruvate dehydrogenase, two regulatory enzymes for glucose use, to be inhibited. In turn, this decreases glucose and increases fatty acid usage in the muscle. There are many benefits of increasing utilization of fatty acids, one being the sparing of muscle glycogen stores. Although muscle glycogen is essential for sudden bursts of speed and muscular force, both glucose and fats can be used at lower levels of exercise intensity, with a benefit of fat having near limitless storage capacities (Oldham et al., 1990). Fat adaptation may also promote greater glycogen storage at rest, with fat adapted horses having higher muscle and liver glycogen levels at rest (Hambleton et al., 1980). These higher levels enable an increased rate of glycolysis during very high intensity exercise.

Indicators of Inflammation and Muscle Damage Following Exercise

Interleukin-1 Beta

Inflammation and disease can cause concentrations of inflammatory cytokines, such as interleukin-1β (IL-1β), to increase due to disruption of the anabolic and catabolic processes. Interleukin-1β is a circulating pro-inflammatory cytokine and plays a crucial role in response to infection and injury (Dinarello, 1996). It is the most distinguished and studied member in the IL-1 family and is produced and secreted by several cell types. However, most studies have only concentrated on its production within innate immune

cells, such as monocytes and macrophages. It originates as the inactive 31 kDa precursor, pro-IL-1β, in response to molecular motifs which are carried by pathogen associated molecular patterns (PAMPs). These PAMPs are responsible for acting on macrophages through the use of pattern recognition receptors, which then regulate the pathways that control gene expression (Takeuchi and Akira, 2010). Inducing pro-IL-1β expression is typically considered to be a priming step, but it not a sufficient secretion stimulus. In order to encourage processing and secretion of active IL-1β, the primed cell needs to come across a PAMP or DAMP (danger associated molecular pattern) (Lopez-Castejon and Brough, 2011). Interleukin-1β is the product of enzymatic cleaving of IL-1 into 2 lower forms, α and β, with IL-1β having 90 times the potency of IL-1α (Lopez-Castejon and Brough, 2011; Palmer and Bertone, 1994). This cleaving process is done by the pro-inflammatory protease, caspase-1. Following primed cell activation, a series of homotypic interactions occur between an adaptor molecule, a cytosolic pattern recognition receptor, and pro-caspase-1, forming an inflammasome, which is an immune system receptor that is formed in response to intracellular pathogens (Xu et al., 2021). This process then activates caspase-1 and promotes secretions of IL-1β (Guo et al, 2015; Schroder and Tschopp, 2010). Considering the close association between IL-1β processing after activation of the inflammasome and its release, interferences that inhibit IL-1β release have done so by solely hindering inflammasome activation which then inhibits formation into the mature form (Brough and Rothwell, 2007). Previous data has shown an intervention that blocks the release pathway of mature IL-1β in response to various NLRP3 activators. Studies have also focused on NLRP3 activation through application of ATP and P2X7 receptor signaling (MacKenzie et al., 2001; Shirasaki et al.,

2014; Brough and Rothwell, 2007; Qu et al., 2007; Andrei et al., 1999) . Interleukin-1β attracts circulating neutrophils to inflammation sites after the release from tissue macrophages, and isolated neutrophil oxidative activity is then directly stimulated in the equine (Benbarek et al., 2008). Only small amounts of IL-1β are needed to create destruction since the receptors are highly sensitive. These receptors can also increase the chance of degradation if they are diseased or inflamed, due to the higher number of receptors. Active at low level concentrations, IL-1β is essential for maintenance of oxidative neutrophil activity and contributes to the continuation of disease (Palmer and Bertone, 1994).

Creatine Kinase

Equine exercise research has concentrated on evaluating physiological capacity and adaptability of horses to different levels of exercise (Malinowski et al., 2002; Krumrych, 2010; Janicki et al., 2013b, 2013c). Overexertion during exercise can result in continuous changes in blood parameters after exercise and may indicate overtraining in horses (Zobba et al., 2011; Padalino et al., 2007). This allows for creatine kinase (CK) activity in the blood to be used to assess adaptions to exercise, as well as a reliable indicator of exercise induced muscle cell damage (Harris et al., 1990; Janicki et al., 2013b). Creatine kinase is an enzyme primarily found in skeletal muscle in horses and can be used as an indicator of muscle damage (Lindholm, 1987). When damage occurs, this enzyme leaks from the cell into blood, causing elevated plasma levels (Noakes, 1987). This enzyme catalyzes the transfer of phosphate from phosphocreatine to adenosine 5'-diphosphate (ADP) to generate cellular adenosino5'-triphosphate (ATP) (Lohmann, 1934). When ATP is regenerated, creatine kinase uses ADP and H^+ to

prevent muscles from acidifying as well as preventing an increase in ADP concentrations. Following intense exercise, CK peaks within 4 to 6 hours and has a half-life of 90 to 120 minutes (Siciliano et al, 1995; Harris et al, 1998; Boffi et al., 2002; Chaney et al., 2004). Studies suggest that collecting blood at least 24 hours after exercise is the most beneficial time to determine skeletal muscle damage. This allows for differentiation between horses that have a normal response versus those that have an abnormal or pathological response after exercise (Harris et al., 1990). Plasma activity of healthy, unexercised horses has been stated to range from 90-275 U/L, with small increases of 4 to 35-fold happening in horses following strenuous exercise (Siciliano et al., 1995; Muñoz et al., 2002; McEwen and Hulland 1986; Valberg et al. 1993). Small short-term increases in serum CK are mostly related to abnormal cell membrane permeability that results from lipid peroxidation and hypoxia of muscle tissues during exercise (Nimmo and Snow, 1982). In one study done by Siciliano et al. (1995), 5 mature, unconditioned, Thoroughbred geldings were used in an initial submaximal exercise test, as well as a final test after 8 weeks of conditioning. The study showed that serum CK levels remained higher than pre-exercise levels from 0-24 hours post exercise. In a second study by Siciliano et al. (1995), 8 mature Thoroughbred horses were used to evaluate effects from short term high intensity exercise and repeated submaximal exercise. Results showed that serum CK levels post exercise were higher in response to the repeated submaximal exercise compared to the short-term high intensity test. Increases in CK activity after exercise is mostly due to changes in the permeability of the cell membrane rather than necrosis of the muscle cells. However, when plasma CK activity reaches 10,000 U/L or greater, this is considered to be an indicator of skeletal muscle disintegration in horses (Siciliano et

al., 1995). These high activity levels are commonly seen in horses with recurrent exertional rhabdomyolysis (RER), which is a hereditary disorder of calcium metabolism. This can result in muscle cramps and stiffness, which may elevate serum CK activity 10 to 900-fold (Castejon et al., 2006; Chaney et al., 2004).

Due to these responses to exercise, the equestrian community seeks to modify the diets of their horses to reduce inflammation and metabolic stress following exercise. This is commonly supplied in the form of oils that are high in essential fatty acids.

Nutritional Modifications to Reduce Inflammation

Dietary Fats

Two methods for increasing the fat content of the diet are to purchase commercial feeds with added fats or to purchase fats separate and top dress onto the basal diet. Regarding the latter, oil supplementation provides a highly digestible source of energy that plays an important role in many metabolic pathways in the body (Crandell et al., 1999).

After consumption of fat, partial digestion starts to occur in the stomach through the digestive enzyme gastric lipase. From there, the remainder of this fat moves to the duodenum of the small intestine, where it is then broken down and absorbed (Doreau and Chilliard, 1997). In other species, bile is stored and released from the gallbladder; however, since horses do not have a gallbladder, bile, along with bile salts, is released from the liver into the small intestine to allow for emulsification of fat globules (Caylor, 1952; Frape, 1998). After emulsification, the pancreas releases a lipase enzyme that attacks these emulsified fats for further breakdown and digestion, resulting in two free fatty acids and a 2-monoglyceride which then create micelles (Doreau and Chilliard,

1997; Frank et al., 2004). These micelles are then absorbed by intestinal mucosal cells via passive diffusion, and the two fatty acids and the monoglyceride are then reassembled in the endoplasmic reticulum. From there they are joined with cholesterol esters and phospholipids to create chylomicrons, which are lipoproteins that carry triglycerides. Lastly, these chylomicrons are released into the lymphatic system where they enter the blood stream and are able to transport lipids throughout the body (Doreau and Chilliard, 1997).

Lipids in the equine diet, found as triglycerides, phospholipids, glycolipids, or sterols, are the predominant energy source during rest and low intensity exercise, and due to their density can provide sustained energy over several hours (Hallebeek and Beynen, 2002; Geelen et al., 1999). Fats are stored as triglycerides, which are hydrolyzed to diglycerides, monoglycerides, and free fatty acids. They are the main storage lipid in plants, therefore making them the primary fat source in the diet (Hallebeek and Beynen, 2002). Triglycerides and diglycerides are polar, non-swelling lipids that are not soluble in water, while monoglycerides and phospholipids are polar, swelling lipids that are not soluble in water. Due to their swelling capacity, these molecules have the means to readily solubilize in micelles when in contact with water (Shiau, 1981). Triglycerides consist of a glycerol backbone attached to three fatty acids (Bayly, 2014). Each fatty acid carbon chain is unbranched and can contain anywhere from 2 to 28 carbons, with 12 to 22 carbon chains being the most prevalent in common feedstuffs (Warren and Vineyard, 2013). The number of carbon atoms present and the placement of double bonds in the carbon chain determine the names of the fatty acids. Saturated fatty acids do not have a double bond in the carbon chain, and this lack of a double bond causes their tails to be

straight and inflexible, which makes them a solid at room temperature. Monounsaturated fatty acids contain only one double bond in the carbon chain, while polyunsaturated fatty acids contain more than one double bond in the carbon chain. An increasing number of double bonds enables increased flexibility in the tails and because of this, both mono- and polyunsaturated fatty acids are liquid at room temperature and are more ideal for fat supplementation (O'Connor et al., 2004). The amount of saturation can influence digestibility, as well as palatability of fats. Research has shown that the degree of unsaturation is positively, but not linearly related to digestibility (Freeman, 1983). This is one of the reasons why saturated fatty acids are not commonly used for fat supplementation in the equine diet. Fats in the diet can also supply omega-3 and omega-6 fatty acids, the two families of essential fatty acids. These must be obtained through the diet since the body does not naturally produce them (O'Connor et al., 2004). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two of the three main omega-3 fatty acids, both of which have been shown to decrease production of inflammatory cytokines (Calder, 2010). Alpha-linolenic acid (ALA), another important omega-3 fatty acid, can be converted to EPA and DHA, however, the double bond addition by the Δ6-desaturase enzyme is the rate-limiting step. This conversion is competing with linoleic acid (LA) being converted to arachidonic acid (AA), since they use the same enzyme, therefore resulting in lower conversion rates of ALA to EPA and DHA (Calder, 2013). However, it has been shown that an increase in LA intake does not increase production of AA, even though LA is a substrate for synthesis of AA (Innes and Calder, 2018). While research is limited on conversion efficiency in horses, human studies have shown a rate of 8-20% for ALA to EPA, and 0.5-9% for ALA to DHA

(Stark et al., 2008). Based on these low conversion rates, supplementation of EPA and DHA is recommended (Arterburn et al., 2006). On the other hand, omega-6 fatty acids can promote inflammatory processes in the body, which are essential to initiate repair of damaged tissues. Therefore, to help promote a balance between inflammation and anti-inflammation, a higher omega 3 to 6 ratio is desired (Simopoulos and Robinson, 1999). Inflammation typically occurs due to muscle damage but providing more anti-inflammatory omega-3 fatty acids might reduce muscle damage during exercise or promote a more rapid repair of damaged tissues after exercise (O'Connor et al., 2004).

Additional Components of Rice Bran Oil with Potential Benefits

Gamma Oryzanol

Gamma (γ) oryzanol is a well sought-after supplement that has been used in performance horses, dogs, and humans. A naturally occurring substance in rice bran oil, γ-oryzanol is a combination of ferulic acid esters that are created through the esterification of sterols or triterpene alcohols with the carboxylic acid group of ferulic acid (Chodkowska et al., 2018; Wilson et al., 2007). This chemical structure has a significant anti-oxidative effect that is caused by part of ferulic acid, similar to cholesterol, that plays a role in regulating blood glucose and plasma lipid parameters (Vorarat, et al., 2010). Previous research has also shown that γ-oryzanol can decrease CK activity and lactate concentrations post exercise and may have an effect on oxidative stress in Thoroughbred horses (Chodkowska et al., 2018). Gamma oryzanol works to reduce the release of CK by inhibiting reactive oxygen species (ROS), therefore protecting lipid cell membranes from peroxidation and reducing permeability (Buzala et al., 2015).

Vitamin E

Vitamin E is a non-enzymatic antioxidant that is part of an intricate antioxidant defense system and plays an important role in neutralizing free radicals that are produced during exercise (Urso and Clarkson, 2003). Rice bran oil also contains Vitamin E, which when combined with γ-oryzanol can work to combat oxidative stress in the body (Huang, 2003). However, limited research has been conducted towards the effects of supplementation of rice bran oil in the equine diet. Vitamin E is the most common antioxidant supplement used for horses (Williams and Carlucci, 2006). The main antioxidant feature of vitamin E is its ability to prevent oxidation of membrane phospholipids, which in turn avoids cellular damage and oxidative stress (Williams and Carlucci, 2006). In addition, vitamin E can have immunomodulatory attributes that influence cytokine response to higher intensity exercise. While vitamin E is ample in fresh forages and readily accessible to horses on pasture, it becomes unstable when baled and stored as hay. To address this issue, many commercial feed companies include vitamin E in their feeds, especially those for performance horses who tend to experience more oxidative stress due to a high intensity workload. The National Research Council (NRC) dietary guidelines for horse's states that in some levels of exercise, supplementing vitamin E in higher amounts than what is currently recommended may improve the status of vitamin E (Ott, 1978). Previous research supports this statement, showing that increased levels of supplemental vitamin E preserved plasma alpha-tocopherol concentrations in intensely exercising racehorses (Rey et al., 2013), endurance horses, and horses used to conduct general exercise tests (Williams and Carlucci, 2006).

In summary, horses undergoing more intense exercise are likely to be subjected to larger amounts of systemic inflammation, which can be determined through biomarkers such as lactate, glucose, IL-1β, and CK. Feeding fats such as rice bran oil and flaxseed oil may help to reduce these inflammatory processes and contribute to weight gain and body condition.

CHAPTER III

Materials and Methods

Horses and Diets

All procedures and animal use in this study were approved by the Sam Houston State University Institutional Animal Care and Use Committee (Protocol Number: 20-01-28-1042-3-01). Twelve healthy Quarter Horses aged 2 yr (± 0.5) were stratified into control (CON; n=4), rice (RICE; n=4), or flax (FLAX; n=4) for a 60-d study. Horses were dewormed with ivermectin (Zimecterin, Boehringer Ingelheim, Duluth, GA) at 3-wk prior to day 0, and then started a 1-wk acclimation period to receiving concentrate (Safechoice Original, Cargill Animal Nutrition, St. Paul, MN) at 0.7% of their BW daily, which approximated 40% of DE requirements. During acclimation, coastal bermudagrass hay was fed at 1.5 to 2% BW daily. Following acclimation to the barn and concentrate feeding, horses were weighed and diets adjusted to feed 60% of DE requirements from hay and 40% from concentrate (NRC, 2007). An acclimation to oil inclusion started 14-d prior to day 0 (Fig.1). Horses fed an oil treatment had 25% of their daily calorie requirement replaced with either crude rice bran oil (Riceland®, Stuttgart, AR) , or a flaxseed oil blend (Soybean oil, cold pressed organic flaxseed oil; Animed™, Winchester, KY) (Table 1). All horses were housed in 3.66 m x 3.66 m stalls (Priefert, Mt. Pleasant, TX) and were allowed 30 min of turnout time a day on a dry lot. Each horse also participated in a behavior and training class, which consisted of light groundwork 2 to 3 times a week with no riding.

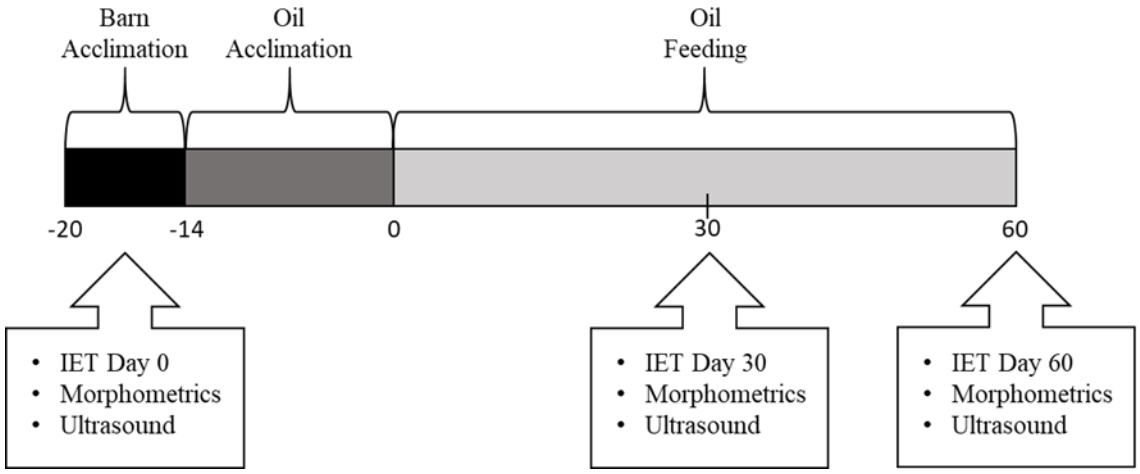


Figure 1. Timeline of data collection in young horses during a 60-d feed trial.

Table 1. Nutrient composition of feeds and oils fed to young horses during a 60-d feeding trial.

Nutrient, DM basis[1]	Hay	Concentrate[2]	RICE[3]	FLAX[4]
DE, MCal/kg	1.83	3.08	-	-
CP, %	10.9	16.3	-	-
ADF, %	36.3	16.7	-	-
NDF, %	52.1	31.2	-	-
EE, %	1.39	6.60	-	-
Ash, %	7.28	1.40	-	-
FA, % of Total EE				
C18:2 n6 (LA)	14.3	50.1	38.4	51.5
C18:3 n3 (ALA)	13.4	4.75	1.47	9.21
C20:5 n3 (EPA)	0	0	0	0
C22:6 n3 (DHA)	1.70	0	0	0
n3:n6 ratio	1.1:1	0.1:1	0.04:1	0.18:1
Calcium, %	0.41	1.92	-	-
Phosphorous, %	0.21	1.17	-	-
Magnesium, %	0.23	0.47	-	-
Potassium, %	1.72	1.27	-	-

[1] Dry Matter (DM); Digestible Energy (DE); Crude Protein (CP); Acid Detergent Fiber (ADF); Neutral Detergent Fiber (NDF); Ether Extract (EE), Fatty Acid (FA); Linoleic Acid (LA); Alpha-Linolenic Acid (ALA); Eicosapentaenoic Acid (EPA); Docosahexaenoic Acid (DHA)
[2]Cargill Animal Nutrition, St. Paul, MN
[3]Crude Rice Bran Oil, Riceland Foods®, Stuttgart, AR
[4]Flaxseed Oil Blend, AniMed™, Winchester, KY

Morphometric Measurements

Measurements were obtained for BW, BCS, cresty neck score (CNS), intermuscular fat (IMF), rump fat thickness (RFT), topline evaluation score (TES), forearm and gaskin circumference, and rib eye area (REA) at 3-wk prior to day 0, and again on day 30 and day 60 (Fig. 1). Body weight was determined using a standard digital livestock platform scale. Body condition score was measured by using the method developed by Henneke et al., (1983). Cresty neck score was measured by using the system developed by Carter et al., (2009). Topline evaluation score was evaluated using

the scoring system developed by Progressive Nutrition (Hopkins, MN). Several measurements were taken via ultrasound by a certified technician, including IMF measured in cm, RFT measured in cm, and REA measured in cm^2. Forearm and gaskin circumference were measured in cm using a soft tape measure around the widest point. Measurements for BCS, CNS, TES, and forearm and gaskin circumference were obtained by trained individuals.

Incremental Exercise Test (IET)

An IET was conducted on an automated horse walker (Priefert, Mt Pleasant, TX) 3-wk prior to day 0, and again on day 30, and day 60 of oil inclusion (Fig. 1). All horses had free choice hay until the start of their exercise test in order to ensure similarities with fed states. The IET was run counterclockwise and consisted of 10 min at 16.1 kph, 2 min at 19.3 kph, 2 min at 22.5 kph, and 2 min at 25.7 kph or until exhaustion. Exhaustion was indicated when the horse struggled to keep pace with the automatic exerciser and the rear panel bumped the horse more than twice. Horses were then hand-walked for 30 min after completion of the exercise test. Blood samples were obtained via jugular venipuncture 4.5 h prior to each exercise test (fasting), and at 1 min, 30 min, 24 h, 48 h, and 72 h post exercise. Blood was then centrifuged for 10 min at 1500 x g, and plasma was aliquoted and stored at -80°C until further analysis. Heart rates were also obtained via stethoscope in the horse's stall directly before the IET, 1 min, and 30 min post on day 0 and 30. On day 60, heart rates were obtained via stethoscope in the horse's stall directly before the IET and 30 min post, with max heart rates obtained via heart rate monitor (KER Clockit Bluetooth HeartRate Monitor, Polar).

Sample Analysis

Plasma from collected blood was thawed at room temperature and analyzed to determine lactate, glucose, IL-1β concentrations, CK activity, and fatty acid percentages.

To determine lactate and glucose concentrations, 100 µL of plasma was pipetted into each well of a 96 well plate and analyzed using a Yellow Springs Instruments (YSI) 2900 Biochemistry Analyzer (Xylem Analytics, Rye brook, New York).

Interleukin-1β concentrations were obtained using the Equine IL-1β ELISA Kit from Kingfisher Biotech Inc. (St. Paul, MN) according to previously published methods (Suagee-Bedore et al., 2017). First, capture antibody was diluted and 100 µL was pipetted into a 96 well plate and incubated at 20°C overnight. After 12 h the capture antibody was dumped, and the plate was blocked with the prepped reagent diluent for 1 h prior to carrying out the protocol. Next, plasma was pipetted into a microcentrifuge tube, diluted 1:2 with reagent diluent, and vortexed. Blocking buffer was then dumped, 100 µL of the diluted plasma samples or standard was pipetted into each well of the plate and incubated at room temperature. After 1 h the plate was dumped, washed 4 times with wash buffer, and tapped against paper towels to remove moisture. Next, 100 µL of diluted detection antibody was pipetted into each well, incubated for 1 h at room temperature, and wash step repeated. Next, 100 µL of diluted streptavidin HRP was pipetted into each well, incubated for 30 min at room temperature, and wash step repeated. Lastly, 100 µL of substrate buffer was pipetted into each well, incubated for 30 min in the dark at room temperature, and then 100 µL of stop buffer was pipetted into each well to stop the reaction. The plate was then read in duplicate at 450nm using a SpectraMax®190 plate reader (Molecular Devices, San Jose, California).

Creatine kinase activity was obtained using the EnzyChrom™ Creatine Kinase Assay Kit from BioAssay Systems. First, 110 µL of water, (10 µL calibrator plus 100 µL of water), and 10 µL of plasma was pipetted into separate wells of a 96 well plate. Next, 100 µL of the reconstituted reagent (10 µL substrate solution, 100 µL assay buffer, and 1 µL enzyme mix for each well) was added into each of the wells containing plasma samples, and the plate was lightly tapped to mix. The plate was then incubated at 37°C inside a SpectraMax®190 plate reader (Molecular Devices, San Jose, California), and read OD340 nm at 20 min and 40 min of incubation. The following equation was then used to calculate CK activity:

$$CK\ (U/L) = (OD_{40min} - OD_{20min} / OD_{Calibrator} - OD_{Water}) \times 150$$

Fatty acid percentages were determined using the protocol created by Perfield et al. (2006) and modified by Corl et al. (2002). First, 100 µL of C17 spike and 3 mL of 3:2 hexane: isopropanol was pipetted into 2 mL of plasma and vortexed. Next, 2 mL of sodium sulfate was then pipetted into the tube and vortexed again, and the upper phase was pipetted out once the phases separated. This upper phase was added to a tube containing 1 g of anhydrous sodium sulfate and vortexed. After sitting at room temperature for 30 min, the solution was transferred (minus the crystals) to a new tube and dried under a stream of nitrogen. Next, 500 µL of hexane and 40 µL of methyl acetate was pipetted into the tube and vortexed. Next, 40 µL of prepared methylation reagent was then pipetted in, vortexed, and allowed to react at room temperature for 24 h. Lastly, 120 µL of termination reagent was pipetted in, and a few grains of calcium chloride added to remove methanol. The remaining upper phase was removed to a GC vial for analysis by gas chromatography (Agilent GC system 6890N, Agilent

Technologies). Obtained peaks were identified using specific markers (Pure Methyl Ester Standards 68D and 91, Nu-Check Prep Inc.), and converted to a percentage of total fatty acids. While chromatographic analysis isolated and quantified multiple fatty acids, data were only analyzed for the following: palmitic acid, oleic acid, linoleic acid, alpha-linolenic acid, eicosanoic acid, eicosatrienoic acid, eicosapentaenoic acid, and docosahexaenoic acid.

Feed and Oil Analysis

Samples for concentrate and hay were analyzed for nutrient and fatty acid concentrations, and samples for both oils were analyzed for fatty acid concentrations (Cumberland Valley Analytical Services, Waynesboro, PA).

Statistical Analysis

All data collected were analyzed using the PROC MIXED procedure of SAS (SAS Enterprise Guide 7.1) with effects for day (0, 30, 60), treatment (CON, RICE, FLAX), time point (pre-exercise, 1 min post, 30 min post, 24 h post, 48 h post, 72 h post), and all interactions using repeated measures ANOVA for time within day with horse as the subject. Data for lactate, glucose, IL-1β, and CK were log transformed to meet normal standards for these parameters, then back transformed as geometric means with a 95% confidence interval. Topline evaluation score data was run in Genmod with a binomial distribution. One FLAX horse was removed from all statistical analysis of lactate, glucose, IL-1β, and CK due to injury prior to the day 60 IET.

CHAPTER IV

Results

Feed Intake

All horses readily consumed their respective diet (CON, RICE, or FLAX) throughout the duration of the 60-d feeding trial (Table 2).

Table 2. Average daily nutrient intake of young horses consuming hay and a pelleted concentrate with no oil (CON) or either crude rice bran oil (RICE) or a flaxseed oil blend (FLAX) replacing 25% of concentrate calories daily for 60-d.

	Treatment		
Nutrient	CON	RICE	FLAX
DE, Mcal	21.58	19.75	19.75
CP, g	1180.93	999.43	999.43
ADF, kg	2.93	2.73	2.73
NDF, kg	5.53	5.13	5.13
EE, g	275.13	400.28	400.28
18:2 n6, g (LA)	101.5	149.25	174.25
18:3 n3, g (ALA)	20.75	19.75	35.75
20:5 n3, g (EPA)	0	0	0
22:6 n3, g (DHA)	1.88	1.88	1.88
Calcium, g	82.92	61.48	61.48
Phosphorous, g	47.41	34.35	34.35
Magnesium, g	27.85	22.66	22.66
Potassium, g	152.93	138.78	138.78

Morphometrics

Body Weight

There were no differences observed for treatment ($P = 0.93$), or treatment by day ($P = 0.77$; Table 3). There was a main effect of day ($P < 0.001$), with lower BW on day 0 (333.6 ± 12.7 kg) than day 30 (357.1 ± 12.7 kg) and day 60 (356.6 ± 12.7 kg). There were no differences observed between day 30 and 60 ($P = 0.98$). The average BW was 352.7 ± 21.7 kg for CON, 342.3 ± 21.7 kg for FLAX, and 352.3 ± 21.7 kg for RICE.

Body Condition Score

No differences were observed for the treatment by day interaction ($P = 0.88$; Table 3). There was a main effect of treatment ($P = 0.05$), with a higher BCS in CON (5.8 ± 0.1) than FLAX (5.4 ± 0.1). There were no differences observed between CON and RICE (5.6 ± 0.1; $P = 0.46$), or between FLAX and RICE ($P = 0.32$). There was also a main effect of day ($P = 0.05$), with tendencies for a lower BCS on day 0 (5.3 ± 0.1) than day 30 (5.8 ± 0.1) and day 60 (5.7 ± 0.1). There were no differences observed between day 30 and 60 ($P = 0.97$).

Cresty Neck Score

Cresty neck score (CNS) had no effect of day ($P = 0.61$) or for treatment by day ($P = 0.16$; Table 3). There was a tendency for effect of treatment ($P = 0.06$), with a higher CNS in RICE (1.8 ± 0.1) than FLAX (1.2 ± 0.1). However, there were no differences observed between CON (1.6 ± 0.1) and FLAX ($P = 0.17$), or between CON and RICE ($P = 0.71$). The average CNS for day was 1.5 ± 0.1 on day 0, 1.4 ± 0.1 on day 30, and 1.6 ± 0.1 on day 60.

Intermuscular Fat

There was no effect of treatment ($P = 0.76$) or treatment by day ($P = 0.16$; Table 3). Intermuscular fat (IMF) had a main effect of day ($P < 0.001$), with measurements being smaller on day 0 (10.5 ± 0.4 cm^2) than day 30 (12.4 ± 0.4 cm^2) and day 60 (12.6 ± 0.4 cm^2). There were no differences observed between day 30 and 60 ($P = 0.84$). The IMF average was 11.5 ± 0.6 cm^2 for CON, 12.0 ± 0.6 cm^2 for FLAX, and 12.1 ± 0.6 cm^2 for RICE.

Rump Fat Thickness

There was no effect of treatment ($P = 0.38$) or treatment by day ($P = 0.86$; Table 3). There was a main effect of day ($P < 0.001$) for RFT, with a significant increase in rump fat thickness (RFT) from day 0 (0.3 ± 0.04 cm) to day 30 (0.5 ± 0.04 cm), and another significant increase in thickness from day 30 to 60 (0.8 ± 0.04 cm). The RFT average was 0.6 ± 0.06 cm for CON, 0.5 ± 0.06 cm for FLAX, and 0.5 ± 0.06 cm for RICE.

Table 3. Mean ± SEM body weight, body condition score, cresty neck score, intermuscular fat, and rump fat thickness in young horses consuming hay[1] and a pelleted concentrate[2] with no oil (CON) or either crude rice bran oil (RICE) or a flaxseed oil blend (FLAX) replacing 25% of concentrate calories daily for 60-d.

Treatment (TRT)	Morphometric Measurements					*P* - Value		
	TRT Mean	D 0	D 30	D 60	SEM	TRT	Day	TRT X D
				--Body Weight (kg)--				
Day Mean	---	333.56[a]	357.08[b]	356.63[b]	12.65			
CON	352.69 ± 21.69	333.98	360.45	363.64	21.90			
RICE	352.31 ± 21.69	337.95	361.14	357.84	21.90	0.93	0.001	0.78
FLAX	342.27 ± 21.69	328.75	349.66	348.41	21.90			
				--Body Condition Score--				
Day Mean	---	5.33[j]	5.75[k]	5.71[k]	0.12			
CON	5.79 ± 0.11[a]	5.68	5.83	5.88	0.22			
RICE	5.61 ± 0.11[ab]	5.25	5.83	5.75	0.22	0.05	0.05	0.89
FLAX	5.38 ± 0.11[b]	5.05	5.60	5.50	0.22			
				--Cresty Neck Score--				
Day Mean	---	1.50	1.42	1.58	0.13			
CON	1.58 ± 0.15	1.75	1.25	1.75	0.23			
RICE	1.75 ± 0.15	1.50	2.00	1.75	0.23	0.06	0.61	0.17
FLAX	1.17 ± 0.15	1.25	1.00	1.25	0.23			
				--Intermuscular fat (cm)--				
Day Mean	---	10.54[a]	12.36[b]	12.55[b]	0.41			
CON	11.46 ± 0.60	10.59	11.47	12.33	0.70			
RICE	12.05 ± 0.60	10.40	12.64	13.11	0.70	0.77	0.001	0.17
FLAX	11.93 ± 0.60	10.63	12.97	12.20	0.70			
				--Rump Fat Thickness (cm)--				
Day Mean	---	0.29[a]	0.51[b]	0.78[c]	0.04			
CON	0.57 ± 0.06	0.32	0.54	0.85	0.07			
RICE	0.54 ± 0.06	0.32	0.54	0.77	0.07	0.39	0.001	0.87
FLAX	0.45 ± 0.06	0.23	0.43	0.70	0.07			

[1]Coastal Bermudagrass fed at 60% of energy requirements

[2]Safe Choice Original (Cargill, St. Paul, MN) fed at 40% of energy requirements

[abc]Means with unlike superscripts differ $P < 0.05$

[jk]Means with unlike superscripts differ $P < 0.1$

Topline Evaluation Score "A"

For the counts of topline evaluation score (TES) "A" there was an effect of time ($P = 0.03$; Table 4), with a tendency for less counts on day 0 (-0.5 ± 0.7) than day 30 (2.2 ± 0.9; $P = 0.07$). No differences were observed between day 0 and 60 (1.6 ± 0.8; $P = 0.16$), or between day 30 and 60 ($P = 0.84$). There was also an effect of treatment ($P = 0.01$), with higher counts in CON (2.9 ± 1.2) than FLAX (-0.5 ± 0.7). There were no differences observed between CON and RICE (0.9 ± 0.7; $P = 0.27$), or between FLAX and RICE ($P = 0.36$).

Forearm Circumference

There was no effect of treatment ($P = 0.81$) or treatment by day ($P = 0.73$; Table 4). A main effect of day ($P < 0.001$) was shown for forearm circumference, with average circumference being lower on day 0 (42.0 ± 0.8 cm) than day 30 (48.8 ± 0.8 cm) and day 60 (48.3 ± 0.8 cm). There were no differences observed between day 30 and 60 ($P = 0.79$). The average forearm circumference was 46.6 ± 1.1 cm for CON, 45.8 ± 1.1 cm for FLAX, and 46.7 ± 1.1 cm for RICE.

Gaskin Circumference

There was no effect of treatment ($P = 0.82$) or treatment by day ($P = 0.76$; Table 4). There was a main effect of day ($P = 0.02$) for gaskin circumference, with average circumference being lower on day 0 (41.0 ± 0.6 cm) than day 30 (42.3 ± 0.6 cm) and day 60 (42.3 ± 0.6 cm). No differences were observed between day 30 and 60 ($P = 0.99$). The average gaskin circumference was 41.4 ± 0.9 cm for CON, 42.0 ± 0.9 cm for FLAX, and 42.0 ± 0.9 cm for RICE.

Rib Eye Area

There was no effect of treatment ($P = 0.32$) or treatment by day ($P = 0.41$; Table 4). There was a main effect of day ($P < 0.001$) for rib eye area (REA), with measurements being smaller on day 0 (72.0 ± 2.4 cm) than day 30 (81.3 ± 2.4 cm) and day 60 (82.3 ± 2.4 cm). There were no differences observed between day 30 and 60 ($P = 0.35$). The REA average was 81.7 ± 4.0 cm for CON, 73.3 ± 4.0 cm for FLAX, and 80.6 ± 4.0 cm for RICE.

Table 4. Mean ± SEM topline score "A", forearm and gaskin circumference, and rib eye area in young horses consuming hay[1] and a pelleted concentrate[2] with no oil (CON) or either crude rice bran oil (RICE) or a flaxseed oil blend (FLAX) replacing 25% of concentrate calories daily for 60-d.

Treatment (TRT)	Morphometric Measurements					*P* - Value		
	TRT Mean	D 0	D 30	D 60	SEM	TRT	Day	TRT X D
				--Topline Score "A" (counts)--				
Day Mean	---	-0.45^{j}	2.18^{k}	1.56^{jk}	0.83			
CON	2.93 ± 1.18^{a}	---	---	---	---			
RICE	0.86 ± 0.72^{ab}	---	---	---	---	0.01	0.03	---
FLAX	-0.50 ± 0.68^{b}	---	---	---	---			
				--Forearm Circumference (cm)--				
Day Mean	---	42.04^{a}	48.79^{b}	48.29^{b}	0.79			
CON	46.63 ± 1.11	43.00	48.75	48.13	1.38			
RICE	46.71 ± 1.11	41.88	48.88	49.38	1.38	0.82	0.001	0.74
FLAX	45.79 ± 1.11	41.25	48.75	47.38	1.38			
				--Gaskin Circumference (cm)--				
Day Mean	---	41.04^{a}	42.25^{b}	42.29^{b}	0.58			
CON	41.42 ± 0.92	40.88	41.88	41.50	1.01			
RICE	41.96 ± 0.92	40.75	42.50	42.63	1.01	0.83	0.02	0.77
FLAX	42.21 ± 0.92	41.50	42.38	42.75	1.01			
				--Rib Eye Area (sq. cm)--				
Day Mean	---	71.97^{a}	81.27^{b}	82.33^{b}	2.38			
CON	81.70 ± 4.03	74.92	83.95	86.24	4.12			
RICE	80.59 ± 4.03	74.04	84.29	83.45	4.12	0.32	0.001	0.42
FLAX	73.28 ± 4.03	66.95	75.58	77.31	4.12			

[1]Coastal Bermudagrass fed at 60% of energy requirements
[2]Safe Choice Original (Cargill, St. Paul, MN) fed at 40% of energy requirements
[ab]Means with unlike superscripts differ $P < 0.05$
[jk]Means with unlike superscripts differ $P < 0.1$

Plasma Fatty Acids

Palmitic Acid (16:0)

There was no effect of treatment ($P = 0.14$) or treatment by day ($P = 0.14$; Table 5). Palmitic acid had a main effect of day ($P < 0.01$), with percentages being higher on day 0 (13.4 ± 0.2 %) than day 30 (12.6 ± 0.2 %) and day 60 (12.5 ± 0.2 %). There were no differences observed between day 30 and day 60 ($P = 0.84$). The average percentage

of palmitic acid was 13.4 ± 0.4 % in CON, 12.4 ± 0.4 % in FLAX, and 12.6 ± 0.4 % in RICE.

Oleic Acid (18:1)

Oleic acid had an interaction for treatment by day ($P < 0.01$), with CON having higher percentages on day 30 (28.6 ± 0.6 %) than day 60 (25.4 ± 0.6 %; Table 5). There were no differences observed between CON on day 0 (27.5 ± 0.6 %) and CON on day 30 ($P = 0.75$), or between CON on day 0 and CON on day 60 ($P = 0.21$). FLAX on day 30 (28.2 ± 0.6 %) had lower percentages than RICE on day 30 (31.4 ± 0.6 %; $P < 0.05$). There were no differences observed between CON (28.6 ± 0.6 %) on day 30 and FLAX on day 30 ($P = 0.99$). There was a tendency for CON on day 30 to have lower percentages than RICE on day 30 (P = 0.07). RICE on day 30 (31.4 ± 0.6 %) had higher percentages than RICE on day 0 (27.0 ± 0.6 %) and RICE on day 60 (27.7 ± 0.6 %; $P < 0.01$). There were no differences observed between RICE on day 0 and RICE on day 60 ($P = 0.99$). The average percentage of oleic acid was 27.0 ± 0.4 % on day 0, 29.4 ± 0.4 % on day 30, and 27.0 ± 0.4 % on day 60. The average percent of oleic acid for each treatment was 27.2 ± 0.5 % for CON, 27.4 ± 0.5 % for FLAX, and 28.7 ± 0.5 % for RICE.

Linoleic Acid (18:2)

Linoleic acid (LA) had an interaction for treatment by day ($P < 0.05$; Table 5), however there were no simple effect differences. The average percentage of LA for each treatment was 48.9 ± 0.5 % in CON, 49.7 ± 0.5 % in FLAX, and 48.9 ± 0.5 % in RICE. The average percentage of LA for day was 48.4 ± 0.4 % on day 0, 49.0 ± 0.4 % on day 30, and 50.0 ± 0.4 % on day 60.

Alpha-Linolenic Acid (18:3)

Alpha-linolenic acid (ALA) had an interaction for treatment by day ($P = 0.02$), with FLAX on day 30 (2.9 ± 0.3 %) having higher percentages than CON on day 30 (1.8 ± 0.3 %) and RICE on day 30 (1.5 ± 0.3 %; Table 5). There were no differences observed between CON and RICE on day 30 ($P = 0.95$). The average percentage of ALA was 2.2 ± 0.1 % on day 0, 2.1 ± 0.1 % on day 30, and 1.9 ± 0.1 % on day 60. The average percentage of ALA for each treatment was 2.0 ± 0.2 % for CON, 2.5 ± 0.2 % for FLAX, and 1.7 ± 0.2 % for RICE.

Eicosanoic Acid (20:1)

There was no effect of treatment ($P = 0.73$) or treatment by day ($P = 0.56$; Table 5). Eicosanoic acid had a main effect of day ($P < 0.001$), with lower percentages on day 60 (0.06 ± 0.08 %) than day 0 (0.6 ± 0.08 %) and day 30 (0.5 ± 0.08 %). There were no differences observed between day 0 and 30 ($P = 0.56$). The average percentage of eicosanoic acid was 0.4 ± 0.1 % in CON, 0.3 ± 0.1 % in FLAX, and 0.4 ± 0.1 % in RICE.

Eicosatrienoic Acid (20:3)

Eicosatrienoic acid had a tendency for an effect of treatment by day ($P = 0.09$), with higher percentages in CON on day 0 (3.1 ± 0.2 %) than CON on day 30 (2.0 ± 0.2 %) and CON on day 60 (2.1 ± 0.2 %; Table 5). No differences were observed between CON on day 30 and CON on day 60 ($P = 1.0$). FLAX on day 0 (3.0 ± 0.2 %) had higher percentages than FLAX on day 30 (1.4 ± 0.2 %) and FLAX on day 60 (1.5 ± 0.2 %). No differences were observed between FLAX on day 30 and FLAX on day 60 ($P = 1.0$). RICE on day 0 (3.3 ± 0.2 %) had higher percentages than RICE on day 30 (1.7 ± 0.2 %) and RICE on day 60 (1.4 ± 0.2 %). No differences were observed between RICE on day

30 and RICE on day 60 ($P = 0.85$). There was an effect of day ($P < 0.001$), with higher percentages on day 0 (3.2 ± 0.1 %) than day 30 (1.7 ± 0.1 %) and day 60 (1.7 ± 0.1 %). No differences were observed between day 30 and day 60 ($P = 0.90$). There was also a tendency for effect of treatment ($P = 0.07$), with a tendency for higher percentages in CON (2.4 ± 0.1 %) than FLAX (2.0 ± 0.1 %). No differences were observed between RICE (2.2 ± 0.1 %) and CON ($P = 0.28$), or between RICE and FLAX ($P = 0.54$).

Eicosapentaenoic Acid (20:5)

There was no interaction for treatment by day ($P = 0.90$; Table 5). Eicosapentaenoic acid (EPA) had a tendency for an effect of day, with a tendency for higher percentages on day 0 (0.3 ± 0.04 %) than day 30 (0.2 ± 0.04 %; $P = 0.06$). There were no differences between day 60 (0.3 ± 0.04 %) and day 0 ($P = 0.65$), or between day 30 and 60 ($P = 0.27$). There was also a tendency for an effect of treatment, with higher percentages in CON (0.3 ± 0.04 %) than RICE (0.2 ± 0.04 %; $P = 0.06$). There were no differences observed between FLAX (0.3 ± 0.04 %) and RICE (P = 0.21), or between CON and FLAX ($P = 0.69$).

Docosahexaenoic Acid (22:6)

Docosahexaenoic acid (DHA) had an interaction for treatment by day, with higher percentages in CON on day 60 (1.4 ± 0.3 %) than FLAX on day 60 (-0.2 ± 0.3 %; $P = 0.03$; Table 5). No differences were observed between RICE (0.9 ± 0.3 %) and FLAX on day 60 ($P = 0.13$), or between RICE and CON on day 60 ($P = 0.96$). There was also a tendency for higher percentages in FLAX on day 0 (1.0 ± 0.3 %) than FLAX on day 60 (-0.2 ± 0.3 %; $P = 0.07$), and a tendency for higher percentages in FLAX on day 30 (1.3 ± 0.3 %) than FLAX on day 60 ($P = 0.06$). No differences were observed between FLAX

on day 0 or 30 (P = 0.99). The average DHA percentage was 1.2 ± 0.1 % for CON, 0.7 ± 0.1 % for FLAX, and 1.0 ± 0.1% for RICE. The average percentage of DHA for day was 1.0 ± 0.2 % on day 0, 1.2 ± 0.2 % on day 30, and 0.7 ± 0.2 % on day 60.

Table 5. Mean ± SEM fatty acid profile of plasma lipids[1] in young horses consuming hay[2] and a pelleted concentrate[3] with no oil (CON) or either crude rice bran oil (RICE) or a flaxseed oil blend (FLAX) replacing 25% of concentrate calories daily for 60-d.

Treatment (TRT)	Plasma Fatty Acid, %					*P* - Value		
	TRT Mean	D 0	D 30	D 60	SEM	TRT	Day	TRT X D
				--16:0--				
Day Mean	---	13.36^{a}	12.60^{b}	12.49^{b}	0.24			
CON	13.44 ± 0.36	13.69	13.35	13.27	0.42			
RICE	12.60 ± 0.36	12.88	12.46	12.46	0.42	0.14	0.004	0.15
FLAX	12.41 ± 0.36	13.50	12.00	11.74	0.42			
				--18:1--				
Day Mean	---	27.00	29.43	26.98	0.35			
CON	27.19 ± 0.46	27.53^{ab}	28.62^{ad}	25.42^{b}	0.61			
RICE	28.73 ± 0.46	27.04^{ab}	31.44^{cd}	27.72^{ab}	0.61	0.08	0.001	0.002
FLAX	27.44 ± 0.46	26.30^{ab}	28.23^{ab}	27.79^{ab}	0.61			
				--18:2--				
Day Mean	---	48.40	49.00	49.98	0.40			
CON	48.87 ± 0.46	48.04	48.29	50.28	0.69			
RICE	48.86 ± 0.46	49.05	47.87	49.65	0.69	0.41	0.03	0.04
FLAX	49.65 ± 0.46	48.10	50.84	50.01	0.69			
				--18:3--				
Day Mean	---	2.18	2.08	1.92	0.12			
CON	2.00 ± 0.15	2.20^{abc}	1.85^{ac}	1.94^{abc}	0.20			
RICE	1.68 ± 0.15	2.00^{abc}	1.52^{a}	1.52^{ac}	0.20	0.007	0.24	0.03
FLAX	2.51 ± 0.15	2.35^{abc}	2.87^{b}	2.30^{abc}	0.20			
				--20:1--				
Day Mean	---	0.59^{a}	0.50^{a}	0.06^{b}	0.08			
CON	0.43 ± 0.09	0.55	0.56	0.17	0.13			
RICE	0.39 ± 0.09	0.59	0.57	0.00	0.13	0.73	0.001	0.57
FLAX	0.33 ± 0.09	0.62	0.35	0.00	0.13			
				--20:3--				
Day Mean	---	3.15^{a}	1.71^{b}	1.67^{b}	0.10			
CON	2.42 ± 0.12	3.12	2.03	2.10	0.17			
RICE	2.15 ± 0.12	3.33	1.70	1.41	0.17	0.07	0.001	0.09
FLAX	1.96 ± 0.12	2.99	1.41	1.49	0.17			
				--20:5--				
Day Mean	---	0.30^{j}	0.15^{k}	0.25^{jk}	0.04			
CON	0.30 ± 0.04^{j}	0.33	0.24	0.34	0.07			
RICE	0.15 ± 0.04^{k}	0.22	0.09	0.14	0.07	0.06	0.07	0.86
FLAX	0.15 ± 0.04^{jk}	0.36	0.13	0.27	0.07			
				--22:6--				
Day Mean	---	0.97	1.15	0.71	0.15			
CON	1.19 ± 0.12	0.98^{ac}	1.24^{a}	1.36^{a}	0.27			
RICE	0.96 ± 0.12	0.98^{ae}	0.95^{ae}	0.94^{ae}	0.27	0.04	0.20	0.03
FLAX	0.69 ± 0.12	0.97^{ae}	1.27^{ae}	-0.17^{bcde}	0.27			

[1]Fatty acids presented as percentage of total lipids.
[2]Coastal Bermudagrass fed at 60% of energy requirements
[3]Safe Choice Original (Cargill, St. Paul, MN) fed at 40% of energy requirements
^{abcde}Means with unlike superscripts differ $P < 0.05$
^{jk}Means with unlike superscripts differ $P < 0.1$

Plasma Metabolites

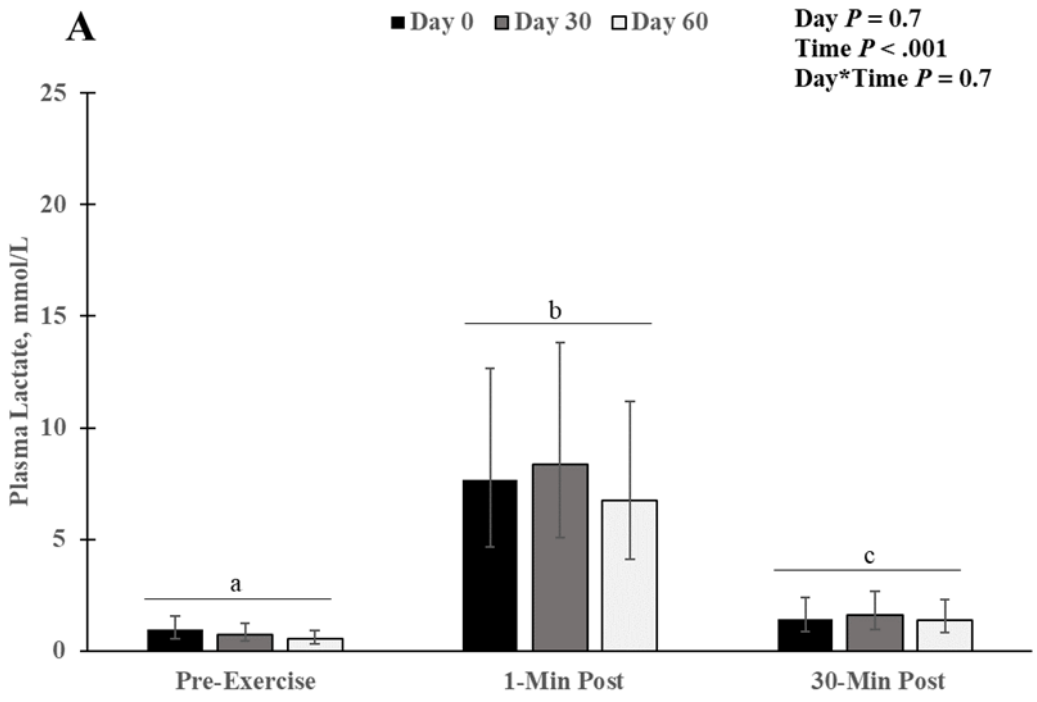
Lactate

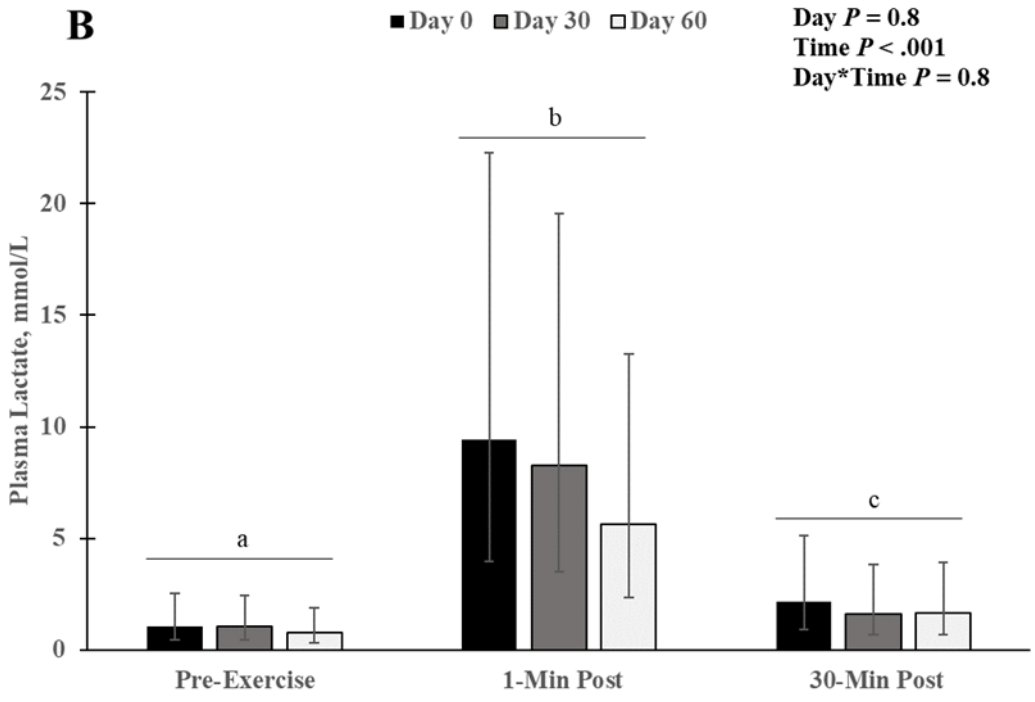
In CON horses there was no effect of day ($P = 0.65$) or day by time ($P = 0.70$; Fig. 2A). There was a main effect of time ($P < 0.001$), with higher geometric mean (95% CI) concentrations at 1 min post exercise (7.6 [5.7, 10.1] mmol/L) than prior to exercise (0.7 [0.5, 1.0] mmol/L) and 30 min post exercise (1.5 [1.1, 2.0] mmol/L), as well as 30 min post exercise having higher concentrations than prior to exercise. Plasma lactate geometric mean concentrations were 2.2 [1.4, 3.3] mmol/L on day 0, 2.2 [1.4, 3.3] mmol/L on day 30, and 1.7 [1.1, 2.7] mmol/L on day 60.

In FLAX horses there was no effect of day ($P = 0.76$) or day by time ($P = 0.79$; Fig. 2B). There was a main effect of time ($P < 0.001$), with higher geometric mean concentrations at 1 min post exercise (7.6 [4.6, 12.5] mmol/L) than prior to exercise (1.0 [0.6, 1.6] mmol/L) and 30 min post exercise (1.8 [1.1, 3.0] mmol/L), as well as 30 min post exercise having higher concentrations than prior to exercise. Plasma lactate geometric mean concentrations were 2.8 [1.2, 6.4] mmol/L on day 0, 2.4 [1.0, 5.5] mmol/L on day 30, and 2.0 [0.9, 4.5] mmol/L on day 60.

In RICE horses there was a tendency for an effect of day by time ($P = 0.09$), with higher geometric mean concentrations on day 0 at 1 min post exercise (10.2 [6.4, 16.3] mmol/L) than prior to exercise (0.7 [0.4, 1.1] mmol/L) and 30 min post exercise (2.4 [1.5, 3.9] mmol/L), as well as higher concentrations on day 0 at 30 min post exercise than prior to exercise (Fig. 2C). On day 30, 1 min post exercise (10.0 [6.3, 16.1] mmol/L) had higher concentrations than prior to exercise (0.7 [0.4, 1.1] mmol/L) and 30 min post exercise (1.4 [0.9, 2.3] mmol/L). There were no differences observed between prior to

and 30 min post exercise on day 30 (P = 0.12). On day 60, 1 min post exercise (7.7 [4.8, 12.3] mmol/L) had higher concentrations than prior to exercise (0.7 [0.4, 1.2] mmol/L) and 30 min post exercise (1.0 [0.6, 1.6] mmol/L). There were no differences observed between prior to and 30 min post exercise on day 60 (P = 0.83). There was also a main effect of time (P < 0.001), with higher geometric mean concentrations at 1 min post exercise (9.2 [7.0, 12.1] mmol/L) than prior to exercise (0.7 [0.5, 0.9] mmol/L) and 30 min post exercise (1.5 [1.2, 2.0] mmol/L), as well as 30 min post exercise having higher concentrations than prior to exercise. There was no effect of day (P = 0.43). Plasma lactate geometric mean concentrations were 2.6 [1.7, 3.9] mmol/L on day 0, 2.2 [1.4, 3.3] mmol/L on day 30, and 1.8 [1.2, 2.7] mmol/L on day 60.




B
Day 0
Day 30
Day 60
Day P = 0.8
Time P < .001
Day*Time P = 0.8
Plasma Lactate, mmol/L
25
20
15
10
5
0
a
b
c
Pre-Exercise
1-Min Post
30-Min Post

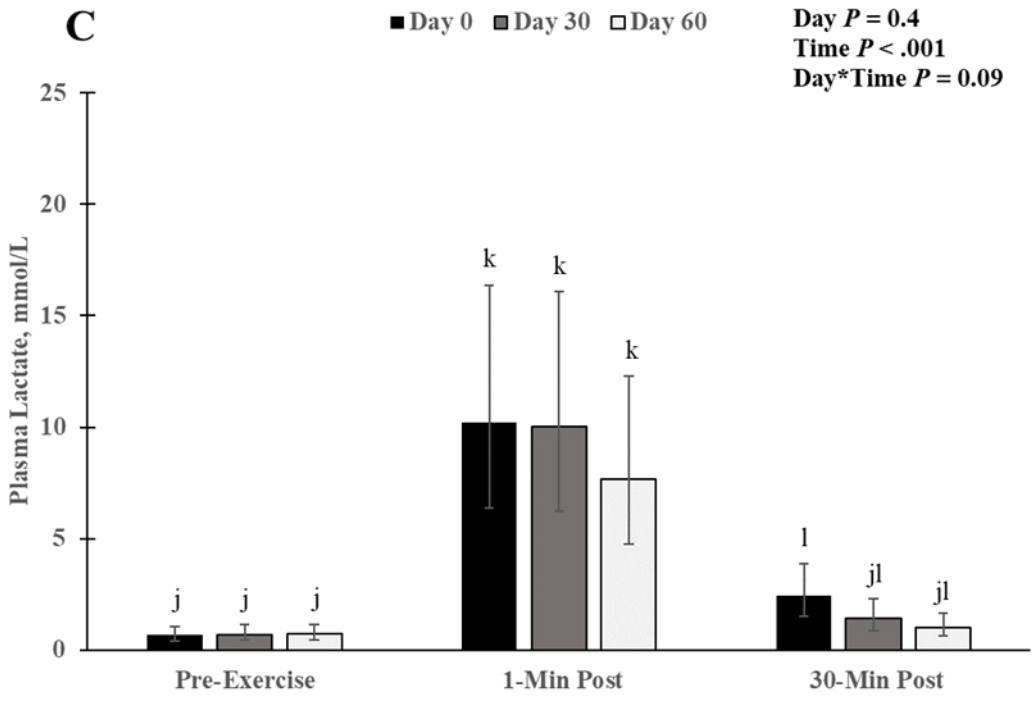

C
Day 0
Day 30
Day 60
Day P = 0.4
Time P < .001
Day*Time P = 0.09
Plasma Lactate, mmol/L
25
20
15
10
5
0
j
j
j
k
k
k
l
jl
jl
Pre-Exercise
1-Min Post
30-Min Post

Figure 2. Mean [LCI, UCI] fasting, 1 min post, and 30 min post exercise plasma lactate concentrations in young horses fed 60% energy from hay (Coastal Bermudagrass) and 40% energy from concentrate (SafeChoice Original, Cargill Animal Nutrition, St. Paul, MN) on days 0, 30, and 60 of a 60-day feeding trial. Where CON (A) did not receive dietary alteration, FLAX (B) had 25% of concentrate calories replaced with a flaxseed oil blend, and RICE (C) had 25% of concentrate calories replaced with crude rice bran oil.
[a,b,c] Means with unlike superscripts differ $P < 0.05$
[j,k,l] Means with unlike superscripts differ $P < 0.1$

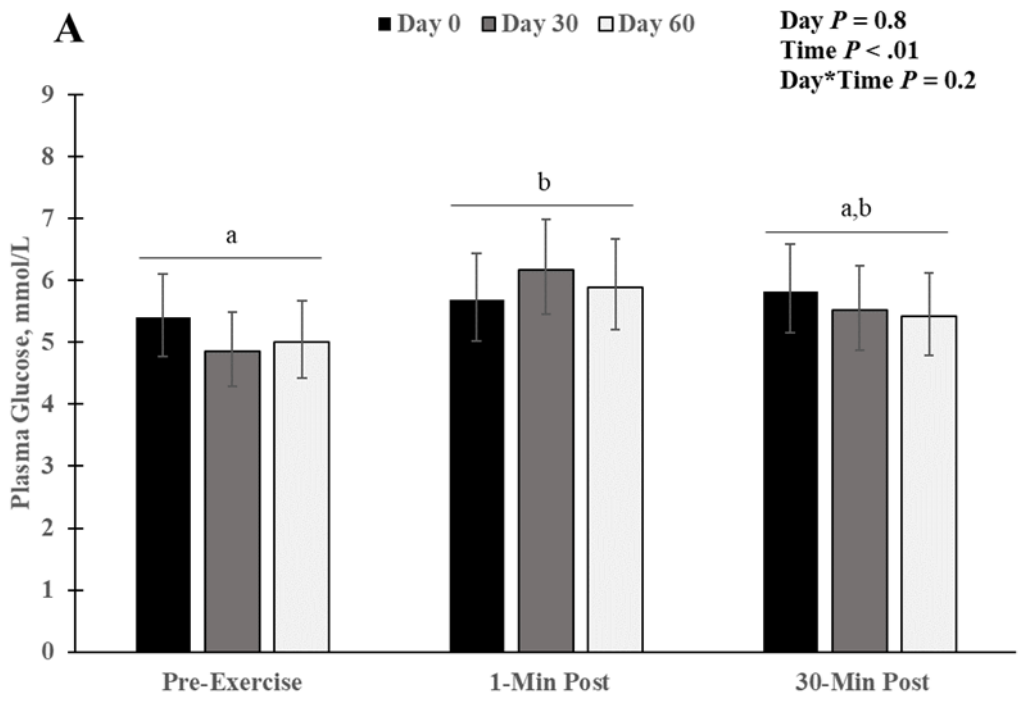
Glucose

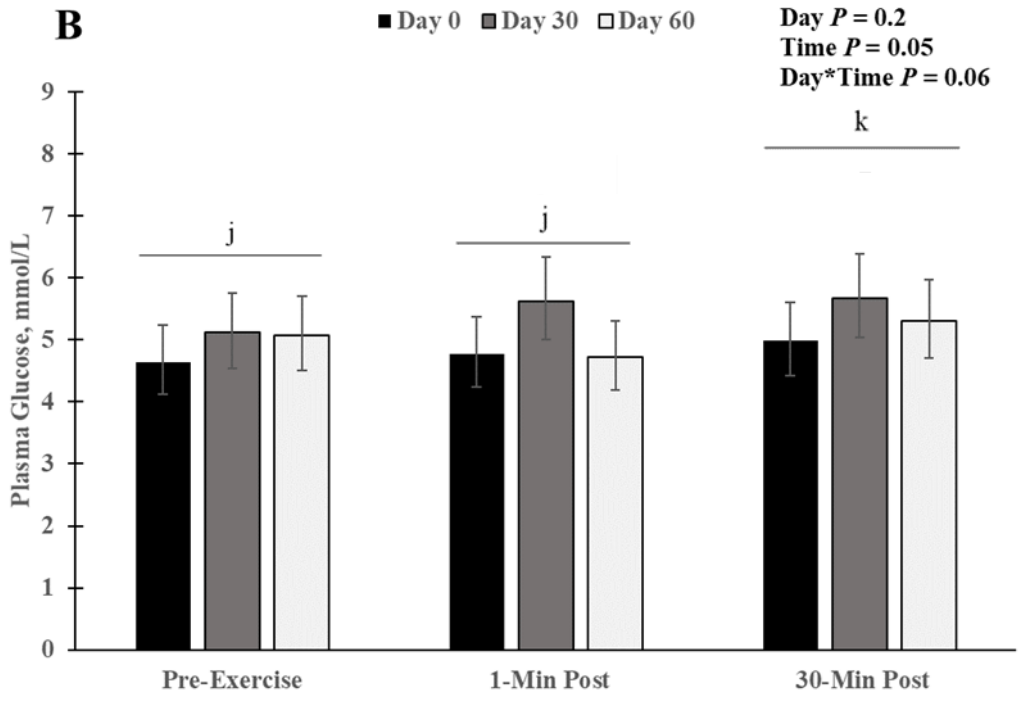
In CON horses there was no effect of day ($P = 0.84$) or day by time ($P = 0.21$; Fig. 3A). There was a main effect of time ($P < 0.01$), with higher geometric mean (95% CI) concentrations 1 min post exercise (5.9 [5.5, 6.4] mmol/L) than prior to exercise (5.1 [4.7, 5.5] mmol/L). There was also a tendency for higher geometric mean concentrations 30 min post exercise (5.6 [5.2, 6.0] mmol/L) than prior to exercise ($P = 0.08$). There were no differences observed between 1 and 30 min post exercise ($P = 0.24$). Plasma glucose geometric mean concentrations were 5.6 [5.1, 6.2] mmol/L on day 0, 5.5 [5.0, 6.1] mmol/L on day 30, and 5.4 [4.9, 6.0] mmol/L on day 60.

In FLAX horses there was a tendency for an effect of day by time ($P = 0.06$; Fig. 3B), however, there were no simple effect differences. There was no effect of day ($P = 0.20$). There was a main effect of time ($P = 0.05$), with a tendency for higher geometric mean concentrations 30 min post exercise (5.3 [5.0, 5.7] mmol/L) than prior to exercise (4.9 [4.6, 5.3] mmol/L) and 1 min post exercise (5.0 [4.7, 5.4] mmol/L). There were no differences observed between prior to and 1 min post exercise ($P = 0.74$). Plasma glucose geometric mean concentrations were 4.8 [4.3, 5.4] mmol/L on day 0, 5.5 [4.9, 6.1] mmol/L on day 30, and 5.0 [4.5, 5.6] mmol/L on day 60.

In RICE horses there was no effect of day ($P = 0.40$) or day by time ($P = 0.82$; Fig. 3C). There was a main effect of time ($P < 0.01$), with lower geometric mean

concentrations prior to exercise (5.0 [4.6, 5.5] mmol/L) than 1 min post exercise (6.1 [5.5, 6.6] mmol/L) and 30 min post exercise (5.8 [5.3, 6.4] mmol/L). There were no differences observed between 1 and 30 min post exercise (P = 0.70). Plasma glucose geometric mean concentrations were 5.4 [4.8, 6.2] mmol/L on day 0, 6.0 [5.3, 6.8] mmol/L on day 30, and 5.4 [4.8, 6.2] mmol/L on day 60.




B
Day 0
Day 30
Day 60
Day P = 0.2
Time P = 0.05
Day*Time P = 0.06
Plasma Glucose, mmol/L
0
1
2
3
4
5
6
7
8
9
j
j
k
Pre-Exercise
1-Min Post
30-Min Post

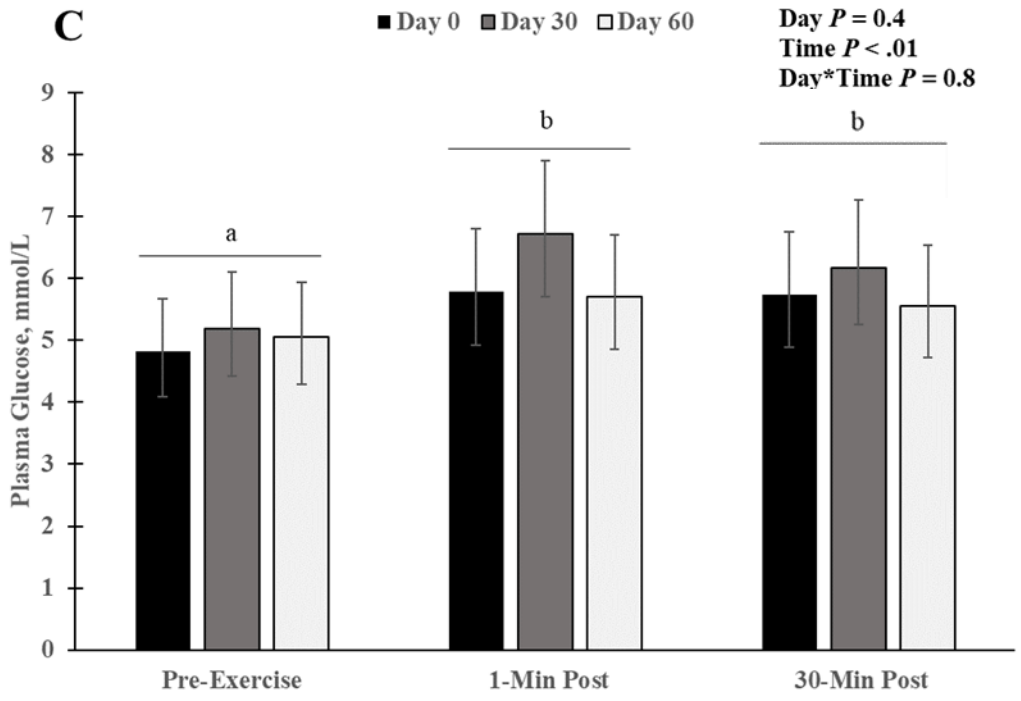

C
Day 0
Day 30
Day 60
Day P = 0.4
Time P < .01
Day*Time P = 0.8
Plasma Glucose, mmol/L
0
1
2
3
4
5
6
7
8
9
a
b
b
Pre-Exercise
1-Min Post
30-Min Post

Figure 3. Mean [LCI, UCI] fasting, 1 min post, and 30 min post exercise plasma glucose concentrations in young horses fed 60% energy from hay (Coastal Bermudagrass) and 40% energy from concentrate (SafeChoice Original, Cargill Animal Nutrition, St. Paul, MN) on days 0, 30, and 60 of a 60-day feeding trial. Where CON (A) did not receive dietary alteration, FLAX (B) had 25% of concentrate calories replaced with a flaxseed oil blend, and RICE (C) had 25% of concentrate calories replaced with crude rice bran oil.
[a,b] Means with unlike superscripts differ $P < 0.05$
[j,k] Means with unlike superscripts differ $P < 0.1$

Heart Rates

On day 0 no differences were observed for heart rate (HR) prior to exercise (P = 0.92), 1 min post exercise (P = 0.15), or 30 min post exercise (P = 0.75; Table 6) between any of the treatments. Prior to exercise, HR was 46.0 ± 4.1 bpm for CON, 44.0 ± 4.1 bpm for FLAX, and 44.0 ± 4.1 bpm for RICE. At 1 min post exercise, HR was 174.0 ± 4.3 bpm for CON, 165.0 ± 4.3 bpm for FLAX, and 161.0 ± 4.3 bpm for RICE. At 30 min post exercise, HR was 62.0 ± 6.6 bpm for CON, 67.0 ± 6.6 bpm for FLAX, and 69.0 ± 6.6 bpm for RICE.

On day 30 no differences were observed for HR prior to exercise (P = 0.93), 1 min post exercise (P = 0.32), or 30 min post exercise (P = 0.94; Table 6) between any of the treatments. Prior to exercise, HR was 44.0 ± 2.2 bpm for CON, 44.0 ± 2.2 bpm for FLAX, and 45.0 ± 2.2 bpm for RICE. At 1 min post exercise, HR was 155.0 ± 5.3 bpm for CON, 159.0 ± 5.3 bpm for FLAX, and 167.0 ± 5.3 bpm for RICE. At 30 min post exercise, HR was 59.0 ± 4.9 bpm for CON, 61.0 ± 4.9 bpm for FLAX, and 59.0 ± 4.9 bpm for RICE.

On day 60 there were no differences observed for HR prior to exercise (P = 0.18), 30 min post exercise (P = 0.88), or max HR (P = 0.34; Table 6). Prior to exercise, HR was 49.0 ± 2.9 bpm for CON, 40.0 ± 3.3 bpm for FLAX, and 44.0 ± 2.9 bpm for RICE. At 30 min post exercise, HR was 57.0 ± 5.6 bpm for CON, 55.0 ± 6.4 bpm for FLAX,

and 53.0 ± 5.6 bpm for RICE. Max HR was 215.0 ± 6.1 bpm for CON, 204.0 ± 7.1 bpm for FLAX, and 218.0 ± 6.1 bpm for RICE.

Table 6. Mean ± SEM pre, 1 min post, and 30 min post exercise heart rates on day 0 and 30, and pre, maximum, and 30 min post exercise heart rates on day 60 in young horses consuming hay[1] and a pelleted concentrate[2] with no oil (CON) or either crude rice bran oil (RICE) or a flaxseed oil blend (FLAX) replacing 25% of concentrate calories daily for 60-d.

	Treatment (TRT)				*P*-Value
Heart Rate (bpm)	CON	RICE	FLAX	SEM	(TRT)
		Day 0			
Pre-Exercise	46.0	44.0	44.0	4.1	0.9
1-Min Post	173.8	161.0	165.0	4.3	0.2
30-Min Post	62.0	69.0	67.0	6.6	0.8
		Day 30			
Pre-Exercise	44.0	45.0	44.0	2.2	0.9
1-Min Post	154.5	166.5	159.0	5.3	0.3
30-Min Post	59.0	59.0	61.0	4.9	1.0
		Day 60			
Pre-Exercise	49.0	44.0	40.0	3.1	0.2
30-Min Post	57.0	53.0	55.0	6.0	0.9
Maximum	215.0	218.0	204.0	6.6	0.3

[1]Coastal Bermudagrass fed at 60% of energy requirements
[2]Safe Choice Original (Cargill, St. Paul, MN) fed at 40% of energy requirements

Indicators of Inflammation and Muscle Damage

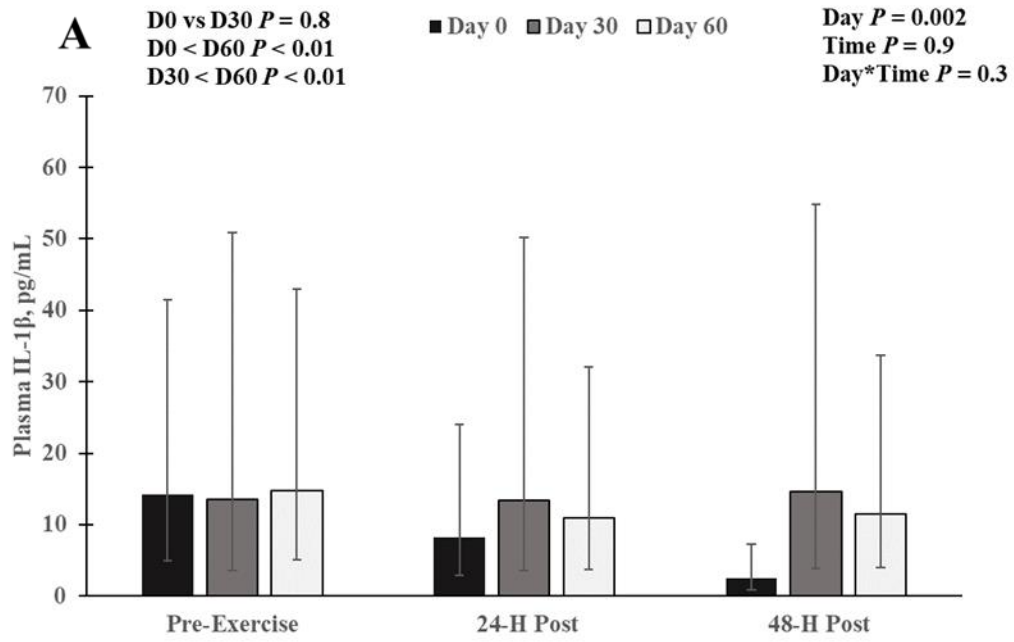
Interleukin-1β

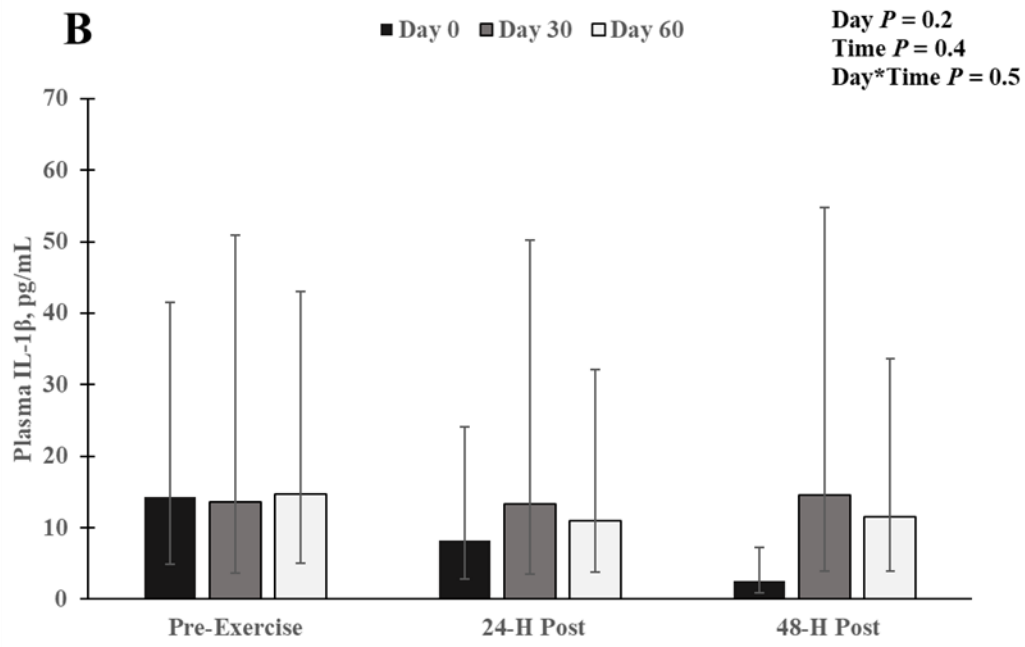
In CON horses there was no effect of time ($P = 0.86$) or day by time ($P = 0.31$; Fig. 4A). There was a main effect of day ($P < 0.01$), with higher geometric mean (95% CI) activity on day 60 (14.0 [7.9, 24.8] pg/mL) than day 0 (3.5 [2.1, 6.0] pg/mL) and day 30 (2.9 [1.5, 5.7] pg/mL). There were no differences observed between day 0 and 30 ($P = 0.87$). Plasma interleukin-1β (IL-1β) geometric mean concentrations were 4.9 [2.8, 8.6] pg/mL prior to exercise, 5.9 [3.4, 10.3] pg/mL at 24 h post exercise, and 4.9 [2.8, 8.7]

pg/mL at 48 h post exercise. One CON horse was removed from day 30 due to being a statistical outlier.

In FLAX horses there was no effect of day (P = 0.21), time (P = 0.36), or day by time (P = 0.46; Fig. 4B). Plasma IL-1β geometric mean concentrations were 6.6 [3.6, 12.3] pg/mL on day 0, 13.8 [6.4, 30.2] pg/mL on day 30, and 12.3 [6.5, 23.0] pg/mL on day 60. For time, concentrations were 14.2 [7.3, 27.6] pg/mL prior to exercise, 10.7 [5.5, 20.8] pg/mL at 24 h post exercise, and 7.5 [3.8, 14.5] pg/mL at 48 h post exercise. One FLAX horse was removed from day 30 due to being a statistical outlier.

In RICE horses there was no effect of day (P = 0.43), time (P = 0.25), or day by time (P = 0.76; Fig. 4C) Plasma IL-1β geometric mean concentrations were 10.3 [8.0, 13.4] pg/mL on day 0, 11.9 [9.3, 15.3] pg/mL on day 30, and 13.4 [10.0, 18.0] pg/mL on day 60. For time, concentrations were 13.1 [10.3, 16.6] pg/mL prior to exercise, 10.1 [7.9, 12.8] pg/mL at 24 h post exercise, and 12.6 [9.8, 16.2] pg/mL at 48 h post exercise.


A
D0 vs D30 P = 0.8
D0 < D60 P < 0.01
D30 < D60 P < 0.01
Day 0
Day 30
Day 60
Day P = 0.002
Time P = 0.9
Day*Time P = 0.3
Plasma IL-1β, pg/mL
70
60
50
40
30
20
10
0
Pre-Exercise
24-H Post
48-H Post


B
Day 0
Day 30
Day 60
Day P = 0.2
Time P = 0.4
Day*Time P = 0.5
Plasma IL-1β, pg/mL
70
60
50
40
30
20
10
0
Pre-Exercise
24-H Post
48-H Post

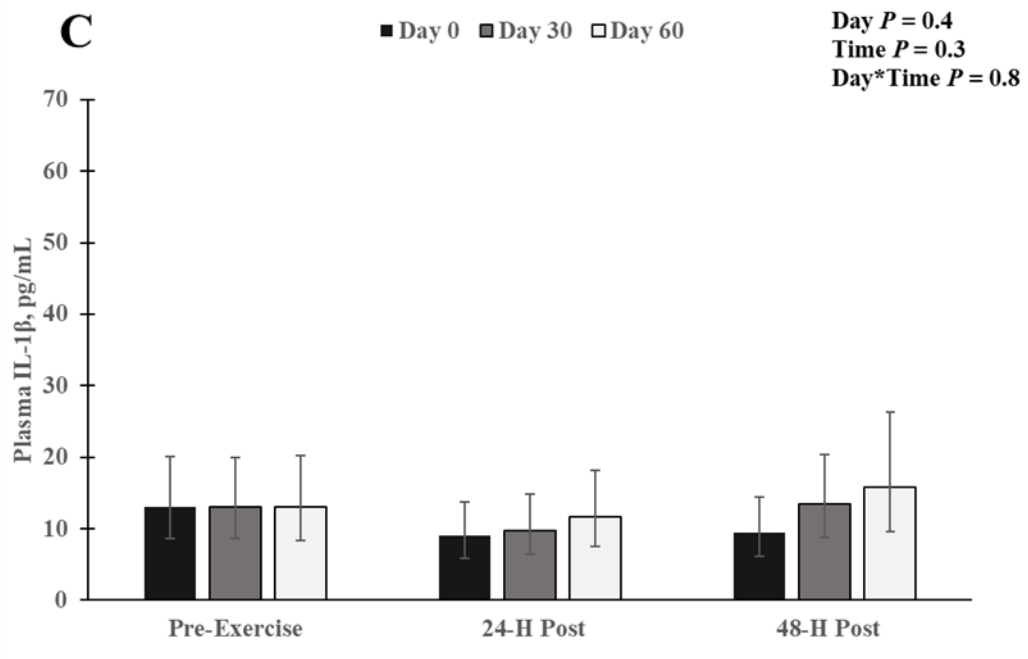


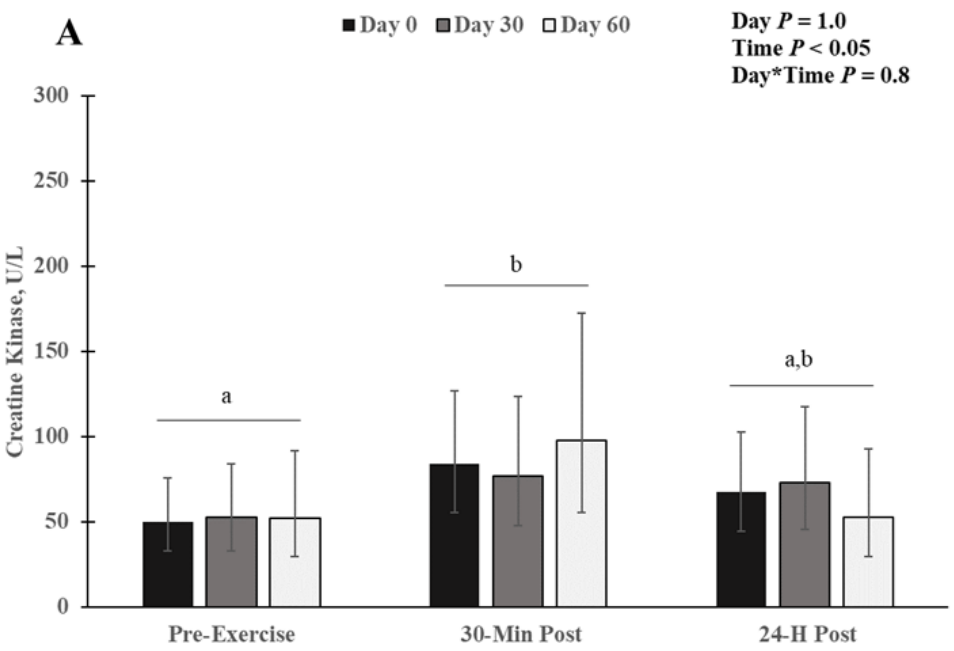
Figure 4. Mean [LCI, UCI] fasting, 24 h post, and 48 h post exercise plasma interleukin-1β concentrations in young horses fed 60% energy from hay (Coastal Bermudagrass) and 40% energy from concentrate (SafeChoice Original, Cargill Animal Nutrition, St. Paul, MN) on days 0, 30, and 60 of a 60-day feeding trial. Where CON (A) did not receive dietary alteration, FLAX (B) had 25% of concentrate calories replaced with a flaxseed oil blend, and RICE (C) had 25% of concentrate calories replaced with crude rice bran oil.

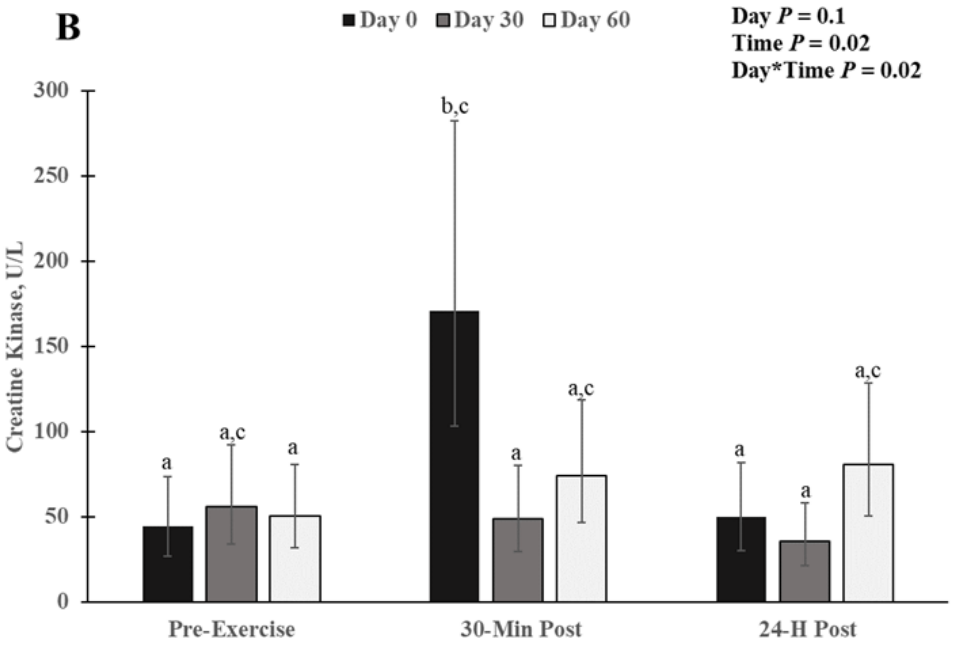
Creatine Kinase

In CON horses there was no effect of day (P = 0.98) or day by time (P = 0.83; Fig. 5A). There was a main effect of time (P = 0.04), with higher geometric mean (95% CI) activity at 30 min post exercise (85.7 [64.9, 113.0] U/L) than prior to exercise (51.5 [39.0, 67.9] U/L). There were no differences observed between prior to and 24 h post exercise (63.8 [48.4, 84.2] U/L; P = 0.48), or between 30 min and 24 h post exercise (P = 0.27). Plasma creatine kinase (CK) geometric mean activity was 65.7 [50.6, 85.2] U/L on day 0, 66.5 [49.9, 88.8] U/L on day 30, and 64.4 [46.1, 89.9] U/L on day 60. Two CON horses were removed from day 60 due to being statistical outliers, one of which was also removed from day 30.

In FLAX horses there was an interaction for day by time (P = 0.01), with higher geometric mean activity on day 0 at 30 min post exercise (170.8 [103.3, 282.1] U/L) than prior to exercise (44.5 [26.9, 73.5] U/L) and 24 h post exercise (49.6 [30.0, 82.0]; Fig. 5B). There were no differences observed between prior to and 24 h post exercise (P = 0.97). There was also a significant difference at 30 min post exercise, with higher activity on day 0 (170.8 [103.3, 282.1] U/L) than day 30 (48.8 [29.7, 80.2] U/L). There were no differences observed at 30 min post exercise between day 60 (74.2 [46.5, 118.3] U/L) and day 0 (P = 0.27), or at 30 min post exercise between day 60 and day 30 (P = 0.91). Plasma CK geometric mean activity was 72.2 [52.1, 100.2] U/L on day 0, 45.9 [33.4, 63.2] U/L on day 30, and 67.1 [51.2, 87.9] U/L on day 60. Mean plasma CK activity for time was 50.1 [38.2, 65.6] U/L prior to exercise, 85.2 [65.1, 111.5] U/L at 30 min post exercise, and 52.2 [39.8, 68.3] U/L at 24 h post exercise.

In RICE horses there was no effect of day (P = 0.10) or day by time (P = 0.16; Fig. 5C). There was a main effect of time (P = 0.02), with higher geometric mean activity at 30 min post exercise (64.3 [50.3, 82.2] U/L) than prior to exercise (39.7 [31.0, 50.7] U/L). There were no differences observed between 24 h post exercise (50.2 [39.3, 64.2] U/L) and prior to exercise (P = 0.35), or between 30 min and 24 h post exercise (P = 0.32). Plasma creatine kinase geometric mean activity was 47.1 [36.1, 61.5] U/L on day 0, 62.6 [49.0, 80.1] U/L on day 30, and 43.4 [33.0, 57.1] U/L on day 60.


A
Day 0
Day 30
Day 60
Day P = 1.0
Time P < 0.05
Day*Time P = 0.8
Creatine Kinase, U/L
300
250
200
150
100
50
0
a
b
a,b
Pre-Exercise
30-Min Post
24-H Post


B
Day 0
Day 30
Day 60
Day P = 0.1
Time P = 0.02
Day*Time P = 0.02
Creatine Kinase, U/L
300
250
200
150
100
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a
a,c
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30-Min Post
24-H Post

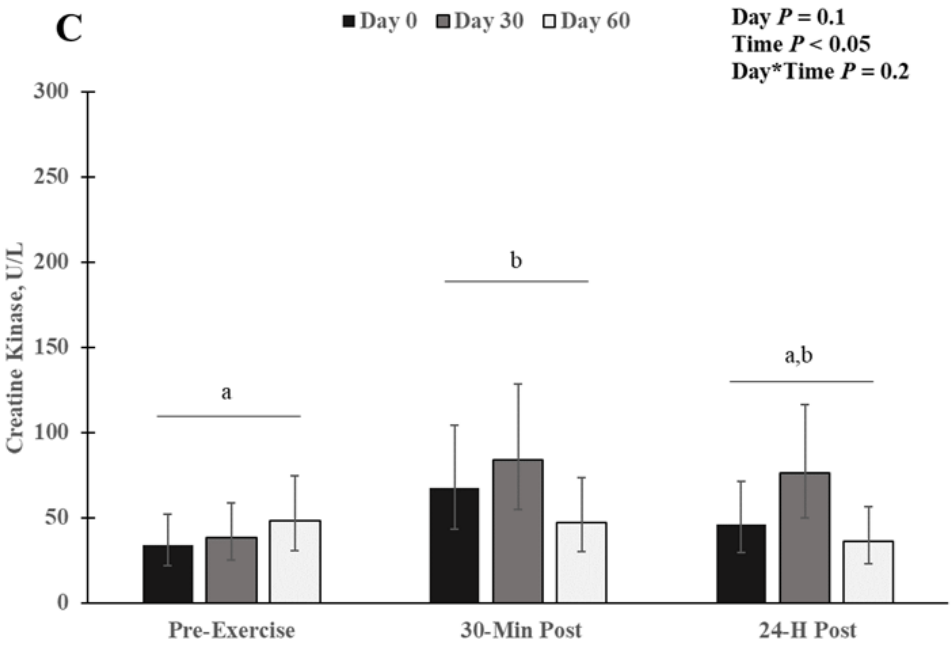

Figure 5. Mean [LCI, UCI] fasting, 30 min post, and 24 h post exercise plasma creatine kinase activity in young horses fed 60% energy from hay (Coastal Bermudagrass) and 40% energy from concentrate (SafeChoice Original, Cargill Animal Nutrition, St. Paul, MN) on days 0, 30, and 60 of a 60-day feeding trial. Where CON (A) did not receive dietary alteration, FLAX (B) had 25% of concentrate calories replaced with a flaxseed oil blend, and RICE (C) had 25% of concentrate calories replaced with crude rice bran oil. [a,b,c] Means with unlike superscripts differ $P < 0.05$

CHAPTER V

Discussion

The main objective of this study was to determine if 60-d of 25% calorie replacement with RICE or FLAX would have an effect on markers of metabolism, muscle breakdown, and inflammation post exercise. Further objectives were to determine the effects of these oils on heart rates during exercise, plasma lipid profiles after replacement, body fat estimates, and muscling scores. We hypothesized that the omega-3 fatty acids in the oils, along with vitamin E and γ-oryzanol in RICE, would lessen muscle damage and inflammation post exercise in the oil fed horses. The main findings of this study were that the exercise test induced anaerobic metabolism in all treatment groups, feeding FLAX increased linoleic acid (LA) and alpha-linolenic acid (ALA) percentages while decreasing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and replacing 25% of concentrate calories with oil did not negatively impact muscle and fat parameters.

During the course of this study BW increased and did not differ by treatment, which supports normal growth patterns in young horses receiving adequate nutrition. Similarly, Dawson et al. (1945) reported that normal growth in horses occurred from birth to 18 mo of age when provided with a diet meeting nutritional recommendations. It has also been shown that horses tend to be at 50% of their mature BW at 1 yr of age, and at 75% of their mature BW at 2 yr of age (Crampton, 1923). Since long yearlings and 2 yr old horses were utilized for this research during this time frame, this could indicate that the horses on the current study had the potential to add roughly 25% of their mature BW during the time frame of this project. Additionally, BW comes from fat and muscle, along

with other components not measured in the current study. Both of our body fat parameters, BCS and rump fat thickness (RFT), increased during the first 30-d in all treatments, which indicates that horses in all three treatments accrued body fat. Horses increased from 9.9% body fat to 12.3% body fat, which according to previous studies suggests that these horses increased from the low end to the high end of the normal body fat range, with no effect of treatment (Westervelt et al., 1976; Kearns et al., 2002). We also observed increases in both forearm and gaskin circumference from day 0 to 30, indicating that the increase in BW from day 0 to 30 was not only due to the addition of fat, but also included muscle accretion. This could have been due in part to the role of daily exercise. It is important to note that while RICE and FLAX had 25% of concentrate calories replaced with oil, therefore removing crude protein and other nutrients supplied in concentrates, horses on those treatments did not differ in any muscle parameters as compared to CON. In summary, all horses increased in BW and body fat, as well as added muscle, indicating that replacing concentrate calories with oil has no negative effects on fat and muscle parameters.

Flaxseed oil has been shown to consist of roughly 50% ALA, which makes it one of the richest sources of omega-3 fatty acids (Klatt, 1986). For the current study, the finding that plasma ALA percentages were higher in FLAX than in CON and RICE on day 30 may indicate that 30-d of FLAX inclusion can help to increase ALA in plasma. Our results agree with those of Hansen at al. (2002), in which horses fed flaxseed oil exhibited greater plasma ALA than control horses at 8 and 12 wk of oil inclusion. As a comparison, Hansen's study added an additional 10% of the horse's energy requirements as flaxseed oil, whereas for the current study 10% of the total energy requirement came

from a flaxseed oil blend. It is important to note that the flaxseed oil fed in Hansen's study was 44% ALA, while our flaxseed oil blend was 9.2% ALA. The RICE horses consumed 58% of the amount of ALA consumed by FLAX horses, and it appears this was not a sufficient quantity to induce alterations to plasma content.

The increased ingestion of ALA did not correlate to increased percentages of longer chain omega-3 fatty acids. As shown in previous research, the conversion of ALA to EPA and DHA is limited due to competition with LA being converted to arachidonic acid (AA). Both of these fatty acids share the Δ6-desaturase enzyme, with ALA being the preferred substrate (Calder, 2013). However, since LA is more prevalent in the diet, metabolism of omega-6 fatty acids takes precedence over omega-3 fatty acid metabolism. This results in lower conversion rates of ALA to EPA and DHA (Calder, 2013). Even after supplementation of ALA, studies have shown that there was little to no evidence of increases in circulating EPA and DHA in horses (Hansen et al, 2002; Vineyard et al., 2010; Hess et al., 2012). For the current study, decreases in EPA after 30-d, as well as observations of CON horses having greater EPA and DHA than RICE and FLAX throughout the study could also be explained by the higher amounts of ALA in the FLAX and RICE treatments, causing a decrease in the conversion rate of ALA to EPA and DHA. The increase in LA from day 0 to 30 could also be explained by the 25% calorie replacement with FLAX, which has also been shown to be about 15% LA. With this, there is an increase in the consumption of LA as well. Hansen et al. (2002) also found that in the horses fed flaxseed oil, LA in plasma was greater than in the control fed horses at 4, 8, and 12 wk of oil inclusion. This indicates that 30-d of feeding an LA rich diet, can increase consumed amounts of LA.

In the current study, the incremental exercise test (IET) was shown to induce anaerobic exercise, as indicated through changes in plasma glucose, lactate, and heart rates. Lactate concentrations, as a measure of anaerobic threshold, are commonly used to assess the level of fitness in equine athletes (Piccione et al., 2010; Williamson et al., 1996). In a study done by Cabrera et al. (2021), lactate concentrations reached the anaerobic threshold directly after high intensity and maximum intensity exercise and returned to aerobic levels during the recovery period, which was 10 min post exercise. Therefore, the increase above 4 mmol/L in lactate concentrations at 1 min post exercise across all treatments observed in the current study, indicates that each treatment was exercising anaerobically. Piccione et al. (2010) also demonstrated a significant increase in lactic acid after exercise, reaching anaerobic status, with concentrations decreasing by 30 min post exercise. For the current study, RICE lactate concentrations returned to baseline at 30 min post exercise on day 30 and 60, with day 0 remaining higher. However, CON and FLAX remained elevated at 30 min post exercise on day 0, 30, and 60. This could be explained by RICE containing γ-oryzanol, a mixture of ferulic acid esters that have been shown to decrease lactic acid concentrations after exercise (Chodkowska et al., 2018).

In our study, increases in plasma glucose concentrations for CON are consistent with a study from Ferraz et al. (2008), where concentrations rose as the exercise intensity increased. Plasma glucose elevations towards the end of exercise have been related to effects of catecholamines and glucagon on the liver, both increasing glucose release from the liver and decreasing re-uptake of glucose by the liver (Simões et al., 1999), therefore increasing blood glucose concentrations. Some studies have indicated that fat

supplementation improves glucose metabolism during exercise and this has further benefits on performance (Oldham et al., 1989; Eaton et al., 1995; Topliff et al, 1983). It is thought that fat supplementation drives an increase in glycogen stores, or creates a glycogen sparing effect by increasing fat utilization during exercise. These varied results may be due to a difference in fat type and amount, treatment duration in relation to metabolic adaptations, or a washout period being utilized (Orme et al., 1997). During recovery, low free fatty acid concentrations may result in glucose being redirected for energy production. Therefore, providing a high fat diet may spare muscle glycogen stores by increasing the availability of lipids during the recovery phase (Hyyppä et al, 1997; Pösö and Hyyppä, 1999). This could be used to explain why our results showed that FLAX and RICE also had increased glucose concentrations post exercise.

Heart rates for this study help to further support that all horses were exercising at an anaerobic level. On day 0 and 30, HR's 1 min post exercise were above the anaerobic threshold of 150-170 bpm (Freeman et al., 2003). On day 60, max HR's for all three treatment groups were also above this range. This suggests that on day 0, 30, and 60 all treatment groups used anaerobic mechanisms.

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that plays an important role in mediating inflammatory responses, and production is often stimulated by strenuous exercise (Lamprecht et al., 2008). In the current study, increases in IL-1β concentrations after 30-d are similar to those seen in a study done by Fikes et al. (2021), where IL-1β showed no changes in the beginning of the study but increased as time and training continued, most notably increasing after a strenuous bout of exercise. Studies have suggested that the most effective option to inhibit the secretion of IL-1β is to inhibit the

activity of caspase-1, the protease that produces mature IL-1β from its precursor protein (Lopez-Castejon and Brough, 2011). Yan et al. (2013) showed that in mice, omega-3 fatty acid supplementation helped to inhibit caspase-1 activity as well as IL-1β secretion by inhibiting NLRP3, a cytosolic protein complex responsible for activating caspase-1. Supplemental omega-3 fatty acids have also shown to inhibit the production of IL-1β after an induced inflammatory response in humans (Zgorzynska et al., 2021), as well as in horses (Brennan et al., 2017). In the current study, FLAX and RICE showed no difference in IL-1β concentrations during the 60-d trial as compared to CON that experienced increases. This could be explained by both RICE and FLAX containing high amounts of omega-3 fatty acids. More research is needed to further determine the effects of omega-3 fatty acid supplementation on IL-1β concentrations in exercising horses.

Creatine kinase (CK) concentrations are commonly used as an indicator of muscle damage in horses. Concentrations typically rise after strenuous exercise, and return to baseline by 24 h post exercise, with a lesser rise in plasma CK indicating less muscle damage (Siciliano et al., 1995). For the current study, in FLAX prior to calorie replacement with oil, the increase in CK activity post exercise followed by the decrease corresponds to a study done by Jagrič-Munih et al. (2012), where CK activity increased directly after exercise, then dropped down to pre-exercise values by 24 h post exercise. Observations of lower CK activity for FLAX at 30 min post exercise after feeding 30-d of flax oil could be explained by higher amounts of ALA present in FLAX. In agreement, other studies have shown that CK activity was markedly lower if horses were provided a high fat diet through the use of top-dressed flaxseed or soy-based oils that contain high amounts of omega-3 fatty acids, such as ALA (Ribeiro et al., 2004). This finding is

potentially due to the protective effects of omega-3 fatty acids on cell membranes (McKenzie et al., 2003). This could also explain why CK activity remained high at 24 h post exercise for RICE and CON in the current study, as horses on these treatments consumed lower amounts of ALA than FLAX.

In summary, 30 to 60-d of inclusion of crude rice bran oil or a flaxseed oil blend may benefit lightly worked young horses by reducing training program related increases in interleukin-1β, while only the flaxseed oil blend reduced exercise induced increases in creatine kinase. Results also indicate that neither oil induces loss of muscle mass nor increase in body fat. Additionally, the flaxseed oil blend has the potential to increase plasma omega-3 and omega-6 fatty acids. Future research could potentially determine the effects of a blended combination of these oils and see how it compares in young growing horses vs. mature performance horses.

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VITA

Kayla Mowry

EDUCATION

Sam Houston State University, Huntsville Texas
Master of Science in Agriculture
Expected graduation date - May 14, 2022

Sam Houston State University, Huntsville, Texas
Bachelor of Science in Animal Science
Minor in Equine Science
Graduated - December 8, 2018

RELEVANT EXPERIENCE

Shot In the Dark Mobile Veterinary Services, Tipton, Oklahoma
Veterinary Assistant September 2021 – March 2022

- Duties consist of restraining and pulling blood for coggins on various equine species at the local monthly horse sale, as well as inputting information into the online database.

Conroe Branch YMCA, Conroe, Texas
Equestrian Center Program Assistant February 2013 - April 2021

- Duties consist of constructing lesson plans, and teaching horseback riding lessons to individuals of all ages, including those with physical and mental disabilities. Leading family trail rides, as well as summer camp trail rides, and instructing equestrian summer camps. Other duties include feeding the horses and providing medical care to horses if needed. Responsibilities also consist of filling in for the Equine Director when on medical leave or vacation.

Equine Veterinary Associates, Conroe, Texas
Volunteer/Shadow June 2005 - August 2005

- Duties included observing and assisting the veterinarian with various medical procedures on farm calls and in the clinic.

TEACHING EXPERIENCE

Sam Houston State University, Huntsville, Texas
Equine Science Teaching Assistant

- Foaling Practicum. Spring 2020
- Equine Behavior and Training. Fall 2020, Spring 2021, Fall 2021
- Equine Nutrition. Summer 2021, Fall 2021

- Equine Safety and Handling. Spring 2022

Sam Houston State University, Huntsville, Texas
Stock Horse Team Coach
- Fall 2020 - Spring 2022

RESEARCH EXPERIENCE

- Thesis research: Effects of Crude Rice Bran Oil and a Flaxseed Oil Blend in Young Horses Engaged in a Training Program
- Data collection: morphometric measurements, heart rate collection, jugular vein blood collection to analyze plasma lipids, lactic acid, glucose, malondialdehyde, interleukin-1β, and creatine kinase after exercise.
- Data analysis: lipid extraction from plasma with use of gas chromatography to obtain data, use of assay kits for malondialdehyde, interleukin-1β, and creatine kinase. Use of YSI machine to analyze lactate and glucose. Use of SAS to analyze all data.

ABSTRACTS

Mowry, K., Thomson, T., Morales, C., Smith, R., Fikes, K., Suagee-Bedore, J. Potential Benefits of Rice Bran Oil for Lightly Worked Young Horses. Presented at the 2022 American Society of Animal Science Southern Section Annual Meeting. *Journal of Animal Science*, Volume 100, Issue Supplement_1, April 2022, Pages 13–14.

Gentry, A., **Mowry, K**., Albritton, H., Suagee-Bedore, J. Inflammatory Response Following a Single Bout of Exercise is More Prominent in Young Horses Compared to Older Horses. Presented at the 2022 American Society of Animal Science Southern Section Annual Meeting. *Journal of Animal Science*, Volume 100, Issue Supplement_1, April 2022, Pages 11–12

MEMBERSHIP AND ACTIVITY

- American Society of Animal Science
- American Quarter Horse Association
- American Paint Horse Association
- SHSU Horseman's Association- member (Fall 2015- Fall 2016) and reporter (Fall 2021-Spring 2022)
- SHSU Stock Horse Team- member (Fall 2015-Fall 2016) and coach (Fall 2020-Spring 2022)
- SHSU Pre-Vet Society- member (Fall 2017- Spring 2018)

GRANTS AND AWARDS

- SHSU Department of Agricultural Sciences Ag Development grant - $500 requested and awarded (Fall 2020 and 2021)
- SHSU School of Agricultural Sciences Ag Workers Graduate Leadership and Service Award – nominated (Spring 2021)
- SHSU Graduate School Travel grant - $500 requested and awarded (Spring 2022)
- SHSU Graduate School Scholarship - $1000 (Spring 2022)
- SHSU Graduate School 3-Minute Thesis Finalist - $100 (Spring 2022)

CONFERENCES ATTENDED

- American Society of Animal Science, Southern Section 2022, Fort Worth, TX