# INDICES OF STRESS IN EXERCISING HORSES FED DIETS CONTAINING VARYING AMOUNTS OF OMEGA-6 AND OMEGA-3 POLYUNSATURATED FATTY ACIDS

A Thesis

by

ALICIA DAWN HOWARD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Animal Science

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Approved by:

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| Chair of Committee, | Gary D. Potter |
| Members, | Pete G. Gibbs |
| | Brett Scott |
| | David M. Hood |
| Head of Department, | Gary Acuff |

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## ABSTRACT

Indices of Stress in Exercising Horses Fed Diets Containing Varying Amounts of Omega-6 and Omega-3 Polyunsaturated Fatty Acids.

(August 2005)

Alicia Dawn Howard, B.S., Texas A&M University

Chairs of Advisory Committee: Dr. Gary D. Potter

Dietary omega-3 polyunsaturated fatty acids have shown substantial benefits in humans including lowered serum cholesterol, blood pressure and indices of stress. The caloric and extracaloric benefits of feeding fat supplemented diets to performance horses are well documented (Webb et al., 1987; Meyers et al., 1989; Julen et al., 1995). However, omega-3 polyunsaturated fatty acids have not been studied to any great extent. This study was conducted to determine the efficacy of feeding omega-3 fatty acids on indices of stress and serum cholesterol in horses.

Nine three- and four-year old horses were assigned to diet treatments according to sex, age and athletic ability. Concentrate diets consisted of: control (A), fat-supplemented diet with corn oil (B) and fat-supplemented diet with extruded/expelled soybean oil (C; N-3). Overall, heart rates were lower in horses fed the fat-supplemented diets compared to the control diet. On reining and cutting exercise days, heart rates were lower ($P<.05$) in horses fed fat-supplemented diets vs. the control diet. There were no differences ($P>.05$) in heart rates during exercise on reining and cutting days between horses fed the two fat-supplemented diets. Recovery heart rates following the SET from

the end of exercise to 60 minutes recovery (R), were significantly quicker in horses fed diet C.

Plasma cortisol concentrations were lowest in horses fed the soy oil-supplemented diet and highest in horses fed the corn oil-supplemented diet. Across treatments, plasma cortisol concentrations during the SET rose due to the onset of exercise and remained significantly higher ($P<.05$) than baseline during the SET. Serum cholesterol concentrations were higher in horses fed corn oil-supplemented diets than in the control or the soy oil-supplemented diets.

There was no significant change ($P>.05$) in body weight between horses consuming these three diets. However, when compared to consuming diets B and C the horses fed diet A had higher ($P<.05$) concentrate intakes. There was no significant difference in hay intake ($P>.05$) between horses consuming the three diets.

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# CHAPTER I

## INTRODUCTION

Popularity in arena performance events has continued to increase in the past decade. Both internationally and in the United States, status and monetary rewards reflect growth & popularity of equine sporting events. Top riders have winnings of more than 2.5 million dollars in their respective events, with elite horses having winnings in excess of several hundred thousand dollars. This can be directly attributed to such events as the National Reined Cow Horse Association, National Reining Horse Association and the National Cutting Horse Association futurities, which now offer purses of $150,000.

For horses to be competitive in events such as three-year-old futurities, intense training regimens must begin at an early age. The National Reined Cowhorse Futurity requires three-year-olds to perform several go-rounds in three different events: reined work, herd work and fence work. The National Cutting Horse Futurity requires maiden three-year-olds to work cattle out of the herd for 2 minutes without rein cues form the rider, and the National Reining Horse Association requires three-year-olds to perform sliding stops, spins, rollbacks and lead changes while being ridden one-handed in a curb bit. The intricacy of maneuvers and levels of work that these young horses are asked to perform causes trainers to push horses hard at younger and younger ages.

Increased levels of training regimens means an increase in stress on juvenile horses. Prolonged periods of elevated stress can be detrimental to the training of these young horses by causing lowered immune response and breakdown in their bodies. As

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trainers and owners ask more from these young athletes, susceptibility to mental and physical breakdowns increases. It is crucial to find ways to keep these horses sound and healthy amidst such demanding careers.

Work loads and energy demands of performance horses require elevated dietary sources of digestible energy. Substantial research has demonstrated that performance horses benefit from supplemental fat as a source of energy, and the caloric and extracaloric benefits of feeding fat-supplemented diets to performance horses are well documented (Webb et al., 1987; Meyers et al., 1989; Julen et al., 1995.) Horses require less feed to maintain performance when fed fat-supplemented diets, and require less fat-supplemented concentrate to maintain body condition and weight. In addition to the extra-caloric benefits of fat-supplementation, a muscle glycogen sparing effect has also been reported. Horses consuming fat-supplemented diets have increased resting muscle glycogen concentrations over horses consuming non fat-supplemented diets. Elevated resting muscle glycogen concentrations suggest that more energy is available for anaerobic work. Fat supplementation also increases the utilization of fats as a source of energy during the aerobic phase of exercise. Addition of fats will also lower the heat increment of diets, thus lowering the heat of fermentation in the exercising horse (Wilson et al., 2003).

It is widely accepted and commonly known that there are many benefits of feeding fat-supplemented diets to exercising horses. Thus, most diets designed for performance horses contain some source of added fat. However, little attention has been focused on the composition of the fat. Most fat supplemented diets contain solvent extracted corn oil and soybean oils, which are high in linoleic acid. It may be necessary

to focus on the source and specific fatty acid profile of the supplemented fat. The literature is limited on the comparative value of fats and oils with differing polyunsaturated fatty acid (PUFA) profiles when fed to athletic animals. There are reported benefits to feeding diets with ratios of omega 6 vs. omega 3 PUFA's in the range of 5-10 (Mooney et al., 1998). Several of the reported benefits of feeding diets with workable ratios of omega 6 vs. omega 3 PUFA's have been: lowered inflammatory response, increased arterial function, disease resistance and immune response in dogs, cats, horses and other species. Since performance horses can benefit in many ways from added dietary fat, an in-depth investigation was needed to determine the effects of supplementing exercising horse diets with differing ratios of polyunsaturated fatty acids on the indices of stress, heart rates and cholesterol levels.

Objectives. The objectives of this study were to:

1) Determine the benefits of a diet containing favorable ratios of omega 6 vs. omega 3 PUFA's in relation to indices of stress, heart rates and cholesterol levels in the exercising horse.
2) Determine if feeding different ratios of omega 6 vs. omega 3 PUFA's is beneficial in reducing the indices of stress, heart rates and mean cholesterol levels of athletically stressed horses.

# CHAPTER II

## REVIEW OF LITERATURE

Feeding Omega-3 and Omega-6 Polyunsaturated Fatty Acids. The healthful benefits of increased omega-3 fatty acids in human diets are widely accepted (De Groot, 1998), and thousands of people in the United States are now supplementing their diets with n-3 PUFA's, typically in the form of fish oil pills (Anderson and Fritsche, 2002). In veterinary medicine as well, there is avid interest in the benefits of these fatty acids in such areas as lowered inflammatory response, cardiology and immune response in dogs, cats, horses and other species. Inclusion of high fat ingredients in horse diets has increased dramatically in the last 10 years. The increased caloric density of fat supplemented diets is particularly important for performance horses that have high energy requirements, such as those in the racing, eventing, cutting, reining and working cow horse industries. The caloric and extracaloric benefits of feeding fat supplemented diets to performance horses are well documented (Webb et al., 1987; Meyers et al., 1989; Julen et al., 1995). The most common high fat ingredients are vegetable and corn oil. Supplemental fat is included in horse diets at rates sufficient to produce crude fat (ether extract) levels of 4 to 8% of the concentrate portion of the diet (O'Connor et al., 2001). In recent years, research in human nutrition has focused on the relationship between fatty acid composition of the diet and various health considerations.

The fatty acids most commonly included in the equine diets are synthesized by plants and have the first of two or more double bond six carbons away from the methyl end of the fatty acid. Arachidonic acid (20:4n-6) and linoleic acid (18:2n-6) are the two most common n-6 fatty acids (Wilson et al., 2003). Linoleic acid is the parent fatty acid

of the omega-6 fatty acid family. Linoleic acid is found in high concentrations of corn, soy, safflower and sunflower oils (James et al., 2000). Once ingested by animals, linoleic acid is converted to arachidonic acid through a process of desaturatin and elongation. Dietary linoleic acid is first desaturated by the Δ5 desaturase enzyme to yield arachidonic acid (Brenner, 1987). Another class of fatty acids, which have their first double bond three carbons away from the methyl end of the carbon chain are n-3 fatty acids. These types of fatty acids are produced in small quantities by plants and also by marine plankton (Lin et al., 1982), and since plankton is the ultimate food source of marine fish, n-3 fatty acids can be found in rich amounts in fatty deposits of these types of fish. There is one problem that we face when trying to feed horses this n-3 rich fish oil: Palitability, which gives us reason to find an n-3 rich source that is more readily accepted by horses.

Exercise Induced Stress. As these young horses are preparing for their respective futurities, they are constantly being hauled to shows and various places to work in anticipation that the horse will get more accustomed to working in varied surroundings. Two of the largest walls that trainers run into are stress and soundness. These horses are mentally stressed, not only from being hauled up and down the road, but from the training regimens that they have undergone for the first two riding years of their life. These less "seasoned" horses can also be more excitable at the horse show itself; therefore, increasing the risk of injury to themselves. Anxiety can cause unintended jerks or moves that can cause injury to the animal. Furthermore, these animals are not only mentally stressed, but also physically. It is mandatory that these horses be sound of hoof when they are competing. Trainers and owners today have many options on procedures

to help insure soundness in their horses; however, a majority of these current techniques may be invasive, and the process itself can cause infection or injury to the joint cartilage itself. Therefore, it would be advantageous to find non-invasive and preventative maintenance techniques to treat these horses.

Several reports support findings that omega-3 fatty acids influence immune reactivity by modulating cell function; therefore decreasing the subjects ability to mount an immune response (Mills et al., 1997 and Anderson and Fritsche, 2002). A normal healthy host encounters many microorganisms on a daily basis; however, only occasionally do they cause disease. There have been two reports that dietary omega-3 fatty acids from fish oil have reduced the severity of coccidiosis in chickens (Allen et al., 1996 and Korver et al., 1997). Additionally, decreased dietary omega-6:omega-3 fatty-acid ratios have been shown to reduce the pathology associated with pneumonia infections in pigs (DeGroot, 1998).

Heart Rates. Heart rates are considered to be a useful indicator of how hard a horse is working, consequently a lower heart rate at the same absolute exercise level could indicate that a horse is experiencing a lower relative work load (O'Connor et al., 2001); and therefore, a lower level of stress.

Epidemiological studies suggest that n-3 PUFA's are involved in the prevention of coronary heart disease (Rousseau et al., 1998, De Groot, 1998, Mehta et al., 1998). A study done by Haumann et. al. (1997) showed that 852 men, over 20 years, that were supplemented with diets rich in omega-3 PUFA's had greater than 50% less occurrences of heart disease than those who did not. Rousseau and Mehta both showed that feeding a

diet that was supplemented with omega-3 PUFA's decreased blood pressure, heart rates and lowered contractility. Strong evidence exists that long-chain omega-3 fatty acids can prevent ventricular arrhythmia in several species through effects on ion channels in excitable tissue, reduction in myocardial oxygen requirements and increased energy efficiency (DeGroot, 1998). DeGroot (1998) also links omega-3 PUFA's to having a beneficial effect on blood vessel occlusion. This reduction was attributed to decreased platelet activity, fibrinogen concentrations, pro-inflammatory mediators, and cell growth factors responsible for arterial-wall cell proliferation that causes narrowing of arteries. Omega-3 PUFA's, through the metabolism of eicosapentaenoic acid, result in decreased platelet aggregation and increased vessel dilation.

Cortisol. Increases in plasma cortisol concentrations have been reported to be linearly related to work intensity (Church et al., 1987). An acute stress response induces an increase in plasma cortisol concentrations by activating the hypothalamo-pitutary-adreal axis (Alexander et al., 1996). An increase in plasma cortisol concentration is frequently reported to indicate a stress response to physiological or pathophysiological stimuli, such as transport, exercise, injury or disease (Mills et al., 1997). Injury and or pain, whether acute or sub-clinical, can directly multiply the level of stress that a horse is working under.

Cholesterol. An increased low density lipoprotein cholesterol concentration has repeatedly been shown to be a strong, consistent and independent risk factor for coronary heart disease in Western Populations (Musliner et al., 1988 and Grundy et al., 1990).

# CHAPTER III

## EXPERIMENTAL PROCEDURES

Animals and Pre-Trial Conditioning Period. Nine, three and four-year-old horses were blocked by age and gender and randomly assigned to a triplicated 3X3 Latin square arrangement of diet treatments. The horses had an average weight of 466 kg. Prior to the experiment, a veterinarian examined the horses for health status. The horses were then dewormed, vaccinated and had their teeth floated if necessary. Horses were maintained at the Texas A&M University Equestrian Center following a management protocol as stated in the Animal Use Protocol approved by the Institutional Agricultural Animal Care and Use Committee.

Prior to the experiment, a 21-d conditioning period was conducted to approximate a similar state of fitness and ability in the horses. During this period, the horses were saddled, walked to a covered arena and ridden in gradually increasing aerobic (heart rate less then 150 beats per minute) and anaerobic (heart rate more than 150 beats per minute) workout. On d 1-2, the horses were worked 5 min at a walk, 10 min at a trot and 5 min at a lope dividing this time equally in both directions. On d 3-16, the lope was lengthened to 10 min, and on d 17-21, the lope was lengthened to 20 min. Additionally, a mechanical "cow" was worked on d 6, 7, 8, 10, 11, and 19; and reining maneuvers consisting of spins, rollbacks and stops were performed on d 4, 5, 17, 18, 20 and 21. The work involving the mechanical cow and reining maneuvers was not standardized between horses as it was necessary to work some horses longer than others to elicit a consistent, background level of ability. After the workout, the horses were then walked back to the

barn, unsaddled and rinsed with water. The horses were fed a commercial diet and bermudagrass hay in amounts to maintain constant body weight.

Experimental Period. Following the 21-d conditioning period, all nine horses began the first period of the Latin square. Each period of the Latin square consisted of 28 d of reined cowhorse training and diet adaptation, followed by a standardized exercise test, 7 d of blood collection with a fecal collection on the last 4 days of each collection period. Horses were rested for one week between the periods to allow acute phase protein concentrations (companion study) to return to baseline. The diets consisted of a conventional-control diet (Diet A), a fat-supplemented diet with 10% corn oil (Diet B) and a fat-supplemented diet with 10% extruded/expelled soybean oil (Diet C) (Table 1). The control diet was a grain-based concentrate and bermudagrass hay. The fat-supplemented diets were grain-based concentrates containing 10% corn oil (Diet B) or extruded/expelled soybean oil (Diet C) that was added at the expense of corn and oats. These diets were also fed with bermudagrass hay. The diets were formulated to contain the same nutrient-to-calorie ratios for protein, Ca and P to ensure the same intake of nutrients relative to energy if there was a decrease in feed intake due to adding fat to the diet (Table 2). The horses were maintained in individual 3.66 m X 3.66 m box stalls with free choice access to water, and were fed their assigned rations in two equal feedings per day at 0200 h and 1400 h. Horses were fed at 12 hour intervals. The times of these feedings were designated for the hours of 0200 and 1400 to allow for the horses to eat four hours prior to riding. Horses daylight exposure was also regulated for the reason that hormones with diurnal patterns, such as cortisol, could be monitored at more

consistent times. After the morning feeding, horses remained in the dark until 0600, when the lights would be turned on by a set of electric timers. The group, period, diet feeding schedule can be seen in Table 3. Initial rations were calculated from expected energy requirements and were adjusted at the beginning of each period to maintain constant body weight.

TABLE 1. COMPOSITION OF CONCENTRATE DIETS (%; as fed)

| **Ingredient** | **Control** | **Fat-Supplemented Corn Oil** | **Fat-Supplemented Extruded/Expelled Soybean oil** |
|---|---|---|---|
| | **A** | **B** | **C** |
| **Corn** | 42.1 | 31.6 | 31.6 |
| **Oats** | 42.1 | 31.6 | 31.6 |
| **Soybean Meal** | 8.0 | 18.5 | 18.5 |
| **Molasses** | 5.0 | 5.0 | 5.0 |
| **Ground Limestone** | 1.6 | 1.4 | 1.4 |
| **Dicalcium phosphate** | .2 | .9 | .9 |
| **Vitamin premix** | .5 | .5 | .5 |
| **Trace mineralized salt** | .5 | .5 | .5 |
| **Fat Source** | | 10.0 | 10.0 |

The exercise protocol during the 28-d training period consisted of an aerobic workout on d 1, 3 and 5 of each week, during which the heart rate was limited to 150 beats per minute (bpm) or less. Additionally, on d 2, 4 and 6 an in-depth maneuver workout was performed to elicit anaerobiosis (heart rate above 150 bpm). The aerobic workout consisted of 10 min at a walk, 10 min at a trot and 30 min at a lope (~260 m/min), dividing this time equally between both directions to achieve a total workload of

approximately 4,000 kg:km per day. On d2 and 4 the lope was shortened to 15 min, followed by a prescribed pattern of maneuvers consisting of galloping circles (~390 m/min), spinning and stopping to achieve a total workload of approximately 4,000 kg:km per day. The pattern began by galloping 4 circles one direction and a change of leads, followed by 4 circles in the opposite direction and a second change of leads. Next, instead of closing this circle, the horses were galloped straight down the fence and performed a sliding stop and rollback into the fence. This was repeated until three sliding stops and rollbacks had been completed, and the last time the horse galloped down the fence it was stopped and backed 5 meters. The horses were then spun 4, 360 degree

TABLE 2. CALCULATED COMPOSITION OF DIETS (as fed)

| **Component** | **Control** | **Fat-Supplemented Corn Oil** | **Fat-Supplemented Extruded/Expelled Soybean Oil** |
|---|---|---|---|
| | **A** | **B** | **C** |
| **Digestible Energy** | | | |
| **Mcal/kg** | 3.4 | 3.95 | 3.95 |
| **Crude Protein** | | | |
| **Percent** | 14.6 | 17.2 | 17.2 |
| **g/Mcal** | 43.0 | 43.5 | 43.5 |
| **Calcium** | | | |
| **Percent** | 0.7 | 0.8 | 0.8 |
| **g/Mcal** | 2.1 | 2.02 | 2.02 |
| **Phosphorus** | | | |
| **Percent** | 0.5 | 0.6 | 0.6 |
| **g/Mcal** | 1.5 | 1.5 | 1.5 |
| **N-6/N-3 ratios** | 17.6:1 | 44.5:1 | 10.3:1 |

TABLE 3. SCHEDULED FEEDINGS: GROUPS, PERIODS AND DIETS

| Horse | Period | Diet |
|---|---|---|
| **1A** | 1 | B |
| **2A** | 1 | B |
| **3A** | 1 | B |
| **1B** | 1 | C |
| **2B** | 1 | C |
| **3B** | 1 | C |
| **1C** | 1 | A |
| **2C** | 1 | A |
| **3C** | 1 | A |
| **1A** | 2 | C |
| **2A** | 2 | C |
| **3A** | 2 | C |
| **1B** | 2 | A |
| **2B** | 2 | A |
| **3B** | 2 | A |
| **2C** | 2 | B |
| **3C** | 2 | B |
| **1A** | 3 | A |
| **2A** | 3 | A |
| **1B** | 3 | B |
| **2B** | 3 | B |
| **3B** | 3 | B |
| **3C** | 3 | C |

rotations in each direction. On d 6 the lope was shortened to 15 minutes followed by a 15 minute anaerobic workout with a mechanical “cow”. The horses completed 5, 1 minute bouts with the mechanical cow traveling back and forth across the arena at 200 m/min, and 2, 1.5-min bouts at 245m/min. The total workload was approximately 4,000 kg:km per day. The mechanical “cow” was controlled by a technician, and a prescribed pattern was performed that consisted of the same number of hindquarter turns in each direction. Attention was taken to accurately calculate distances and speed traveled to determine work load. During the weekly exercise routine, the horses were ridden solely by two

riders, in a randomized order, and each rider exercised the horses an equal amount of days each week to standardize the exercise routine. On d 7 of each week the horses were given a day of stall rest. Additionally, on d 26 and 27 of each period the horses were given additional days of stall rest to prepare for a standardized exercise test (SET).

On d 29 of each period the horses were subjected to a SET designed to mimic the stress young horses would experience at a reined cowhorse futurity. The SET was designed to work the horses at an intensity to elicit anaerobiosis, mount a stress response and a sub-clinical inflammatory response (companion study). The SET began with a warm up period that consisted of 10 min at a walk, 10 min at a trot and 5 in at a lope (~260 m/min) followed by 7 min at a gallop (~390 m/min). Horses were then spun 4, 360 degree rotations in each direction followed by 1 min of rest. Horses were then started on one end of the arena, galloped to the other end of the arena (67 m) and brought to a sliding stop. This process was repeated until 4 stops had been completed. Three repetitions of 4 sliding stops were completed equaling a total of 12 sliding stops. Horses were rested 1 min between each repetition of 4 stops. Horses were then worked on a mechanical "cow". Horses completed 3, 1-min bouts with the mechanical "cow" traveling back and forth across the arena at 200 m/min, and 2, 1.5-min bouts at 240 m/min with 1 min rest between each bout. The mechanical cow was controlled by a technician, and a prescribed pattern was performed that consisted of the same number of hindquarter turns in each direction. The SET was designed to achieve a total workload of approximately 4600 kg:km. All horses were ridden by the same rider during the SET to ensure a standardized exercise routine.

Horses were all shod appropriately for reined cowhorse performance before the beginning of each period. In addition to proper shoeing the horses also received routine preventative veterinary care. Each horse had individual saddle pads and saddle girths to minimize any skin inflammation possibly caused by fungus. Additionally, each horse was fitted with bell boots, splint boots and skid boots to minimize contact injuries.

Measurements and Sampling. Change in body weight was monitored during each period and feed intakes were adjusted prior to the start of each period to minimize changes in body weight.

Heart rates were obtained during exercise. V-Maxx heart rate moniters from Equine Performance Technology were used on each horse on d 2, 4 6 (Table 4) and at pre-set times during the standardized exercise test (Table 5). Heart rate monitors were tested prior to being used on horses to standardize their output. Horses were shaved in each spot that the electrodes were attached (Figure 1). Electrodes on each monitor were cleaned with alcohol and primed with elecrotransmitting gel prior to being attached to each horse. Electrodes were attached at the same places on the horse each time. Riders utilized a wrist-worn receiver that allowed them to monitor the changing heart rates. During exercise, riders would call out readings to technicians, for spreadsheet entry. On d 2 and 4 heart rates were obtained while horses were in their stalls, upon arriving to the arena, at the completion of the warm-up, gallop 1$^{st}$ set of circles and change leads, gallop 2$^{nd}$ set of circles and change leads, 4 sliding stops, 8 spins, 30 sec recovery (R), 1 min R, 2 min R, 5 min R and 10 min R. On d 6 heart rates were obtained in the stall, upon arriving to the arena, at the completion of the warm-up, 1$^{st}$ 1 min bout with mechanical

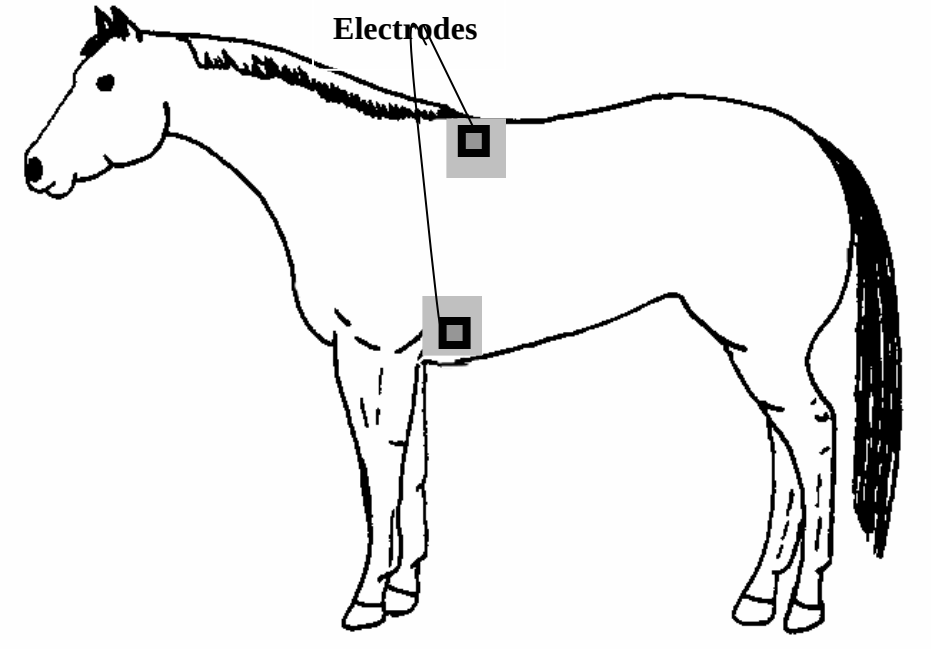


FIGURE 1. HEART RATE MONITOR POSITIONS

cow, 2nd 1 min bout, 3rd 1 min bout, 4th 1 min bout, 5th 1 min bout, 1st 1.5 min bout, 2nd 1.5 min bout, 3rd 1.5 min bout, 30 sec R, 1 min R, 2 min R, 5 min R and 10 min R. During the standardized exercise test, heart rates were obtained in the stall, upon arriving at the arena, at the end of the warm-up, end of circles, end of stops, end of cow, 30 sec R, 1 min R, 2 min R, 5 min R and 10 min R.

TABLE 4. HEART RATE SAMPLING SCHEDULE TAKEN FOR WEEKLY ANALYSIS ON DAYS 2, 4 AND 6

| **Day 2, 4 - "Reining" Workout** | **Day 6 - "Cutting" Workout** |
|---|---|
| Stall | Stall |
| Arena | Arena |
| End of Warm Up (walk, trot, lope) | End of Warm Up (walk, trot, lope) |
| Gallop 4 Circles, Change Leads | 1st 1 min bout on mechanical "cow" |
| Gallop 4 Circles, Change Leads | 2nd 1 min bout on mechanical "cow" |
| End of 4 sliding stops | 3rd 1 min bout on mechanical "cow" |
| End of Spins | 4th 1 min bout on mechanical "cow" |
| 0.5 min recovery (R) | 5th 1 min bout on mechanical "cow" |
| 1 R | 1st 1.5 min bout on mechanical "cow" |
| 2 R | 2nd 1.5 min bout on mechanical "cow" |
| 5 R | 0.5 min recovery (R) |
| 10 R | 1 R |
| | 2 R |
| | 5 R |
| | 10 R |

Venous blood samples were obtained by jugular venipuncture for the harvest of serum using blood collection vacutainer tubes without additive (Table 6). Consideration was taken for the diurnal variations in plasma cortisol concentrations throughout the day; therefore, all weekly plasma blood samples were taken at 1300h, in an effort to reduce the effect of such diurnal variation. Additional venous blood samples were obtained by venipuncture for harvest of plasma using vacutainer tubes containing EDTA (Table 6). Both samples were taken weekly, 30 minutes prior to the afternoon feeding during the training period on d 0, 6, 13, 20, 27 and at pre-set times during the standardized exercise

TABLE 5. HEART RATE SAMPLING SCHEDULE FOR ANALYSIS AT PRE-SET TIMES DURING THE SET

| |
|---|
| Stall |
| Arena |
| End of Warm Up (walk, trot, lope) |
| Gallop 4 Circles, Change Leads |
| Gallop 4 Circles, Change Leads |
| End of 4 sliding stops |
| End of Spins |
| 2nd 1 min bout on mechanical "cow" |
| 3rd 1 min bout on mechanical "cow" |
| 4th 1 min bout on mechanical "cow" |
| 5th 1 min bout on mechanical "cow" |
| 1st 1.5 min bout on mechanical "cow" |
| 0.5 min recovery (R) |
| 1 R |
| 2 R |
| 5 R |
| 10 R |

test via jugular catheters. Following the collection, all blood samples were transported to the lab and placed in the refrigerator. Samples used for the collection of serum and plasma were allowed to clot 1 hour prior to centrifugation. After clotting, serum and plasma samples were removed from the refrigerator and loaded into a centrifuge in such a way that the rotor was balanced. Samples were spun at 2,500 rpm for 30 minutes. After separation, plasma and serum from each collection tube was pipeted into 1.5 ml microcentrifuge tubes, labeled, securely capped and stored in a freezer (-20˚C) until laboratory analysis.

TABLE 6. SAMPLING SCHEDULE FOR THE HARVEST OF BLOOD SERUM FOR ANALYSIS OF CORTISOL, ACTH AND CHOLESTEROL
(Samples collected 30 min prior to afternoon feeding and during SET)

| Day | Time | Samples Taken |
|---|---|---|
| 0 | | * |
| 6 | | * |
| 13 | | * |
| 20 | | * |
| 27 | Stall | * |
| 27 | Arena | * |
| 27 | Mid-point | * |
| 27 | End | * |
| 27 | 0.5 min recovery(R) | * |
| 27 | 1 R | * |
| 27 | 5 R | * |
| 27 | 15 R | * |
| 27 | 60 R | * |
| 27 | 120 R | * |
| 48 | | * |
| 55 | | * |
| 62 | | * |
| 71 | Stall | * |
| 71 | Arena | * |
| 71 | Mid-point | * |
| 71 | End | * |
| 71 | 0.5 min recovery(R) | * |
| 71 | 1 R | * |
| 71 | 5 R | * |
| 71 | 15 R | * |
| 71 | 60 R | * |
| 71 | 120 R | * |
| 90 | | * |
| 97 | | * |
| 104 | | * |
| 113 | Stall | * |
| 113 | Arena | * |
| 113 | Mid-point | * |
| 113 | End | * |
| 113 | 0.5 min recovery(R) | * |
| 113 | 1 R | * |
| 113 | 5 R | * |
| 113 | 15 R | * |
| 113 | 60 R | * |
| 113 | 120 R | * |

Horses were confined to tie stalls for total collection of feces from 1400 h on d 30 to 1400 h on d 34. Rubber mats were placed on individual tie stall floors and the horses were partitioned to the side of the stall by metal panels. Mats were kept swept free of contamination of dirt, shavings and hay to prevent contamination of fecal samples. Individual tie stalls were checked at 15 min intervals during the total collection period. Total fecal collections were accomplished using a hand brush and dust pan, and deposited into labeled tubs. Every 3 hours, feces were weighed using a digital scale and recording the weight in a log book. Ten percent of the collected fecal weight was calculated, recorded and placed in a freezer bag. Freezer bags were placed in a freezer (-20˚C) until laboratory analyses. While confined in stalls, horses were exercised by hand walking each day. Any defecation during exercise was measured and recorded as part of the daily output but not used for laboratory analyses due to the likelihood of contamination. Feed and hay samples were collected once daily during total collection, placed in freezer bags and stored until laboratory analyses.

Laboratory Analyses. Plasma samples were analyzed for cortisol concentrations by Clinical Assays™ GammaCoat™ $^{125}$I Radioimmunoassay kits (DiaSorin, Inc., Stillwater, MN, USA). Blood serum samples were thawed, brought to room temperature and vortexed. All sample, control and standard tubes were labeled. A water bath was prepared by filling it to the required level and heating it to 37˚C and labeled radioactive. Buffer was prepared by combining 1 vial of the cortisol tracer to 1 vial of PBS buffer solution, mixed and stored in a foil-wrapped container. $^{125}$I cortisol tracer-buffer was transferred to the radioactive area. 10 µl Cortisol Serum Blank was pipeted into the

blank tubes. 10 µl of the appropriate standard was pipeted into each of the standard tubes. 10 µl of the appropriate control and samples were pipeted into each of the horse control and unknown sample tubes. Assay tubes were then moved to the radioactive area and 1.0 µl $^{125}$I cortisol tracer-buffer was added to each tube. All tubes were gently vortexed, as to avoid foaming, and placed in the water bath to incubate for 45 minutes. Following the incubation period, the supernatant was decanted from all the tubes except TOTAL by inverting the tubes. Tubes stood inverted on absorbent paper for 25 minutes. Tubes were tapped on absorbent paper to remove any liquid from the rims. Tubes were then transferred into racks that would be placed into the gamma counter. Prior to the racks of samples being run, a background check was performed on the machine. Sample racks were then inserted into the Packard Cobra II Gamma Counter for 1 minute and counted for radioactivity levels.

Plasma samples were analyzed for ACTH using 125I RIA kits (DiaSorin, Inc., Stillwater, MN, USA). Blood serum samples were thawed, brought to room temperature, vortexed and placed on crushed ice. All sample, control and standard tubes were labeled. All tubes and reagents were placed on crushed ice. $^{125}$I ACTH tracer-buffer was transferred to the radioactive area. A water bath was prepared by filling it to the required level and heating it to 37˚C and labeled radioactive. 100 µl of zero standard was pipeted into the NSB tubes. 100 µl of zero standard was pipeted into the zero standard tubes. 100 µl of appropriate standard was pipeted into each of the standard tubes. 100 µl of the appropriate control or sample was pipeted into each of the kit control, horse control and unknown sample tubes. 200 µl of ACTH antiserum was pipeted into all tubes except TOTAL tubes and NSB tubes. Assay was moved to “hot” bench. 200 µl of $^{125}$I ACTH

was added to all tubes. Tubes were then vortexed and incubated for 16-24 hours at 2-8 ˚C. Tubes were then centrifuged for 20 minutes at 2000 rpm at 20-25 ˚C. Following the incubation period, the supernatant was decanted from all the tubes except TOTAL by inverting the tubes. Tubes stood inverted on absorbent paper for 25 minutes. Tubes were tapped on absorbent paper to remove any liquid from the rims. Tubes were then transferred into racks that would be placed into the gamma counter. Prior to the racks of samples being run, a background check was performed on the machine. Sample racks were then inserted into the Packard Cobra II Gamma Counter for 1 minute and counted for radioactivity levels.

Samples were analyzed for cholesterol concentrations by the Texas Veterinary Medical Diagnostic Lab at Texas A&M University.

All laboratory analyses were conducted in duplicate and samples were reanalyzed when necessary to achieve accuracy within 5% error.

Statistical Analyses. Data were analyzed for period, diet, time and diet by time interactions using analysis of variance appropriate for the Latin square design using Stata statistical software. When main differences were established at the $P<.05$ level, differences between diets and time were further analyzed using the Fisher-Hayter means comparison test. Significant differences were established at the $P<.05$ level.

# CHAPTER IV

## RESULTS AND DISCUSSION

Body Weight, Concentrate Intake, Hay Intake and Total Feed Intake. There was no significant difference (P>.05) in horse body weight across the three diets (Table 7). Concentrate intake, hay intake and total feed intake are also shown in table 6. When compared to consuming diet B (4.14 kg) and diet C (4.47 kg) the horses fed diet A (5.21 kg) required significantly more (P<.05) in concentrate to maintain constant body weight. However, there were no differences (P>.05) in concentrate intake between horses fed diets B and C. Hay intake was similar (P>.05) for horses on all three diets. Total feed intake was higher (P<.05) for horses fed diet A (9.05 kg) than for horses fed diet B (7.81 kg). Although numerically higher, total feed intake was not significantly (P>.05) for horses fed diet A when compared to horses fed diet C (8.19 kg). There was no significant difference (P>.05) in total feed intake for horses fed diets B and C (Wilson et al., 2003).

TABLE 7. MEAN WEIGHTS, DAILY INTAKES, DIGESTION OF DRY MATTER, DIGESTION OF ORGANIC MATTER AND CALCULATED DE

| Item | Diet | | | | | |
|---|---|---|---|---|---|---|
| | A | | B | | C | |
| | **Mean** | **SEM** | **Mean** | **SEM** | **Mean** | **SEM** |
| **Weight (kg)** | 1089.75 | 42.44 | 1085.38 | 50.24 | 1075.00 | 36.82 |
| **Concentrate Intake (kg)** | 5.21 [a] | .15 | 4.14[b] | .10 | 4.48[b] | .26 |
| **Hay Intake (kg)** | 3.84 | .22 | 3.66 | .19 | 3.72 | .19 |
| **Total Intake (kg)** | 9.05[a] | .35 | 7.81[b] | .18 | 8.19 [a,b] | .38 |
| **Digested Dry Matter (kg)** | 5.61 [a] | .26 | 4.69 [b] | .21 | 5.17 [a,b] | .17 |
| **Dry Matter Digestibility (%)** | 62.23 | 2.64 | 60.08 | 2.22 | 63.67 | 2.35 |
| **Digested Organic Matter (kg)** | 6.58 [a] | .29 | 5.57 [b] | .19 | 6.15 [a,b] | .22 |
| **Organic Matter Digestibility (%)** | 69.02 | 1.98 | 67.57 | 1.91 | 70.95 | 1.99 |

[a,b] Means lacking common superscript within rows differ (P<.05)

Chemical Analysis of Diets. The fatty acid composition of the fats contained in the diets are shown in Tables A3-A6. The control concentrate, diet A, contained a 17.6:1 ratio of n-6 to n-3 fatty acids. An identical experimental concentrate was used with both fat-supplemented diets. The experimental concentrate contained an 18.5:1 ratio of n-6/n-3 fatty acids. Diet B was supplemented (top-dressed) with corn oil and diet C was supplemented (top-dressed) with mechanically extracted soybean oil. Both oils were added to the experimental diet at 10% of the total weight of the concentrate. The corn oil supplement contained a 58.8:1 ratio of n-6/n-3 fatty acids. When the corn oil fat-supplement was added to the experimental concentrate the concentrate diet had a 44.5:11 ratio of n-6:n-3 polyunsaturated fatty acids. The soybean oil contained a 9.5:1 ratio of n-6/n-3 fatty acids. When the soybean oil was added to the experimental concentrate the concentrate diet had a 10.3:1 ratio of n-6:n-3 polyunsaturated fatty acids.

Heart Rates. On reining and cutting exercise days, heart rates were lower ($P<.05$) in horses fed fat-supplemented diets vs. the control diet. However, there were no differences ($P>.05$) in heart rates during exercise on reining and cutting days between horses fed the two fat-supplemented diets. Additionally, there were no significant ($P>.05$) differences in heart rates due to diet during the exercise portion of the SET. This is explained by the observation that all horses reached near maximal heart rates during the SET. Recovery heart rates following the SET from the end of exercise to 60 R, were significantly lowest in horses fed diet C (Figure 7). Maximum heart rates were achieved following the stopping part of the SET (Figure 6). There was also a trend of lower heart

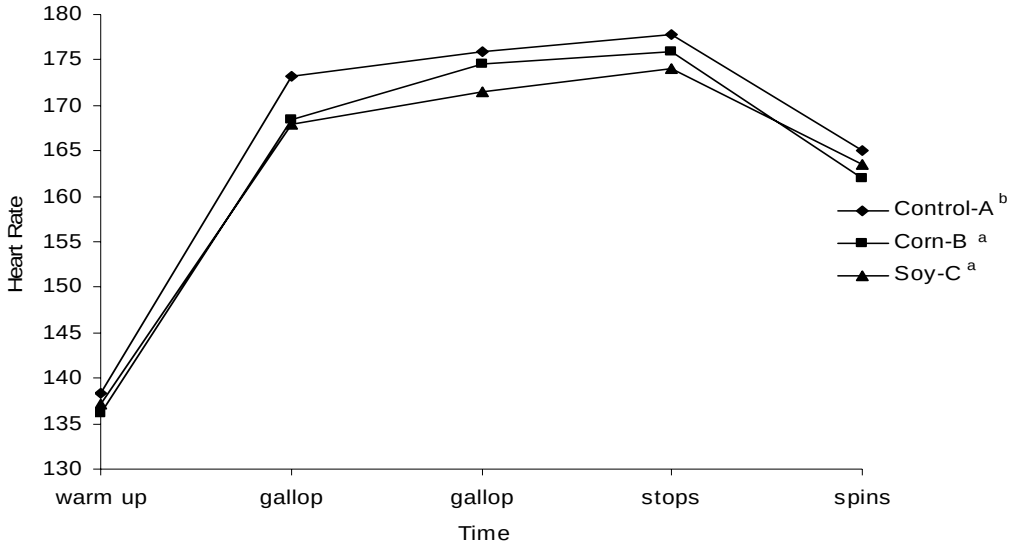


FIGURE 2. EFFECT OF DIET ON MEAN HEART RATES FOR EXERCISE PORTION OF REINING WORKOUT

[a,b]Groups lacking common superscripts differ (P<.05)

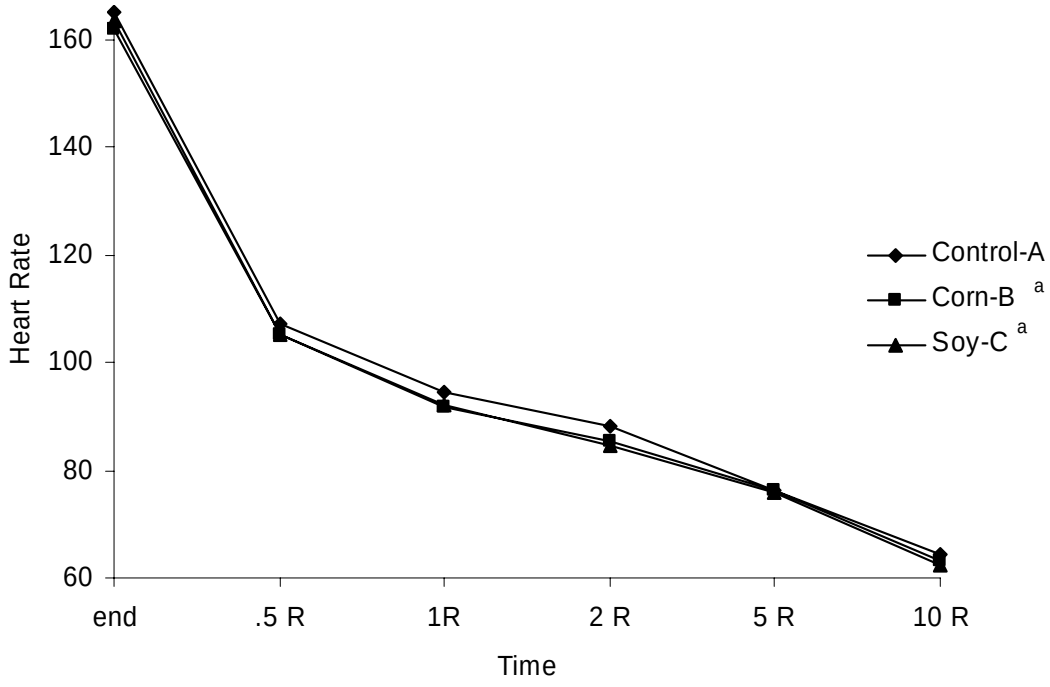


FIGURE 3. EFFECT OF DIET ON MEAN HEART RATES FOR RECOVERY PORTION OF THE REINING WORKOUT

[a,b]Groups lacking common superscripts differ (P<.05).

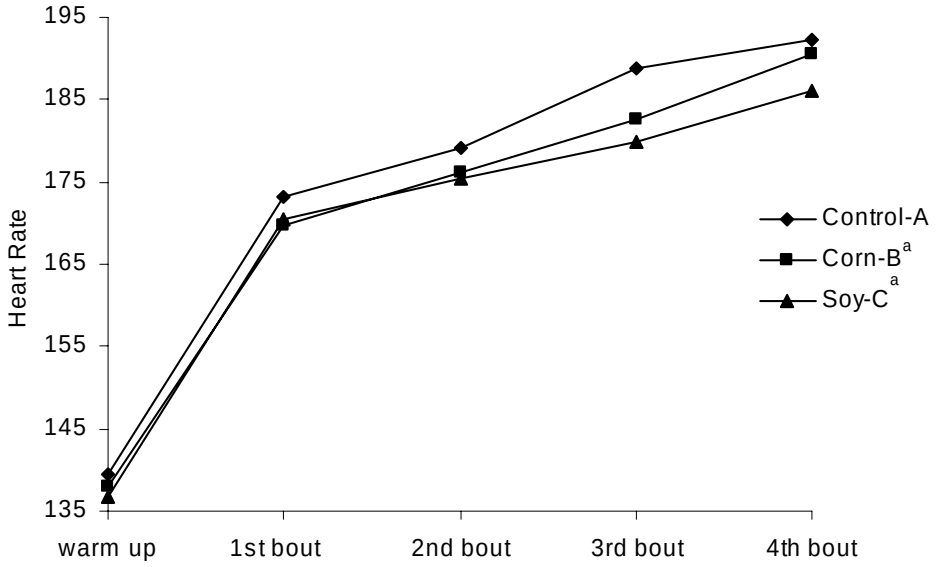


FIGURE 4. EFFECT OF DIET ON MEAN HEART RATES FOR EXERCISE PORTION OF THE CUTTING WORK

[a,b]Groups lacking common superscripts differ (P<.05).

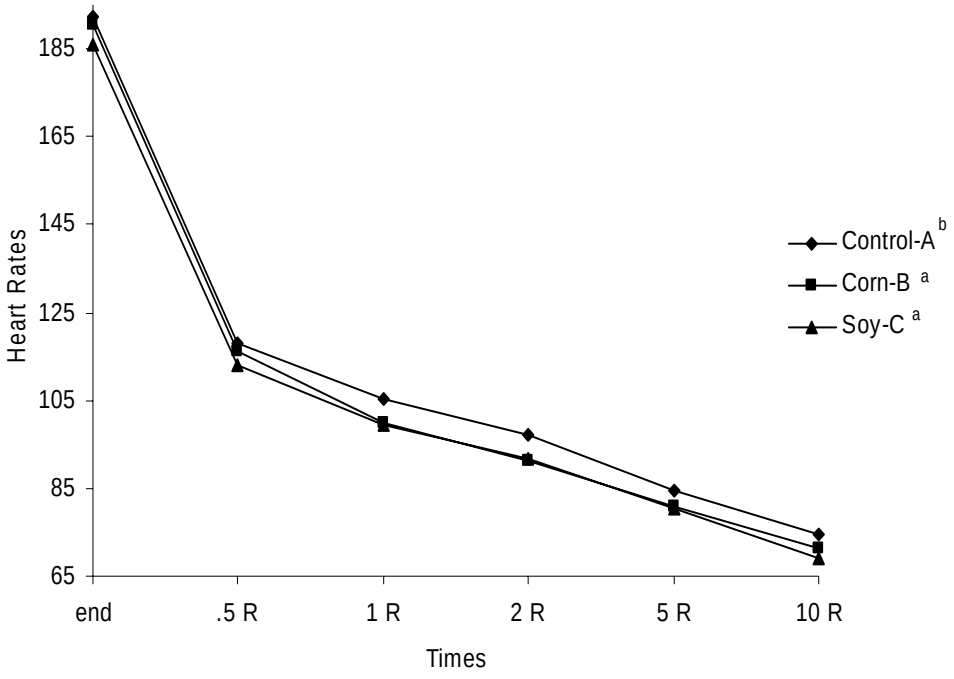


FIGURE 5. EFFECT OF DIET ON MEAN HEART RATES FOR RECOVERY PORTION OF THE CUTTING WORK

[a,b]Groups lacking common superscripts differ (P<.05).

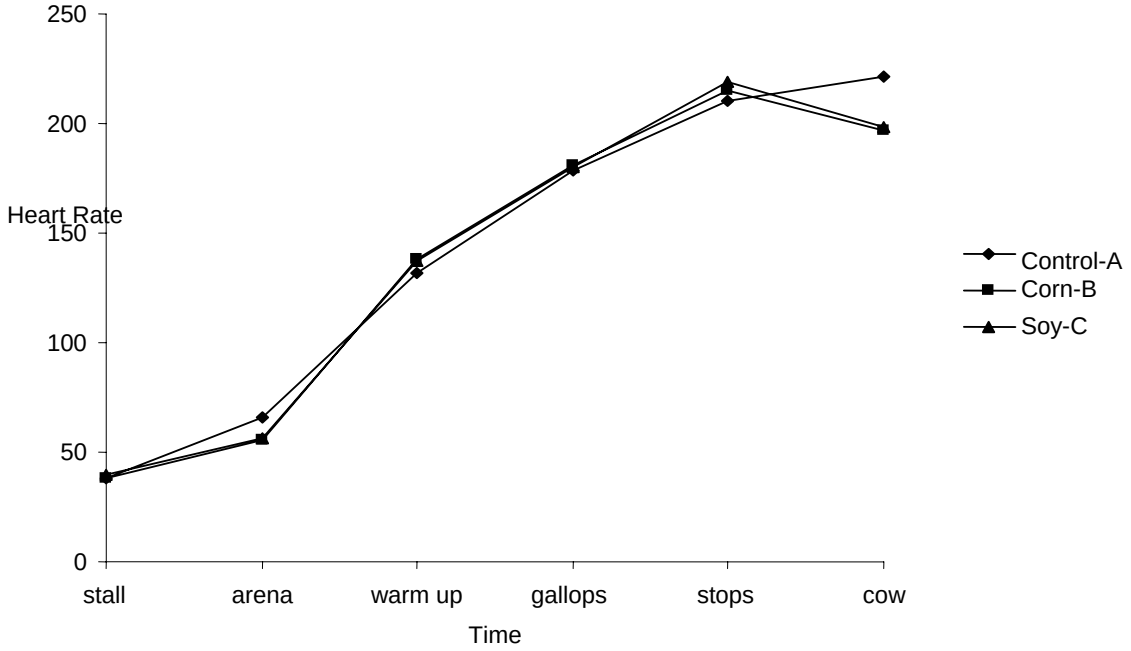


FIGURE 6. EFFECT OF DIET ON MEAN HEART RATES FOR EXERCISE PORTION OF SET

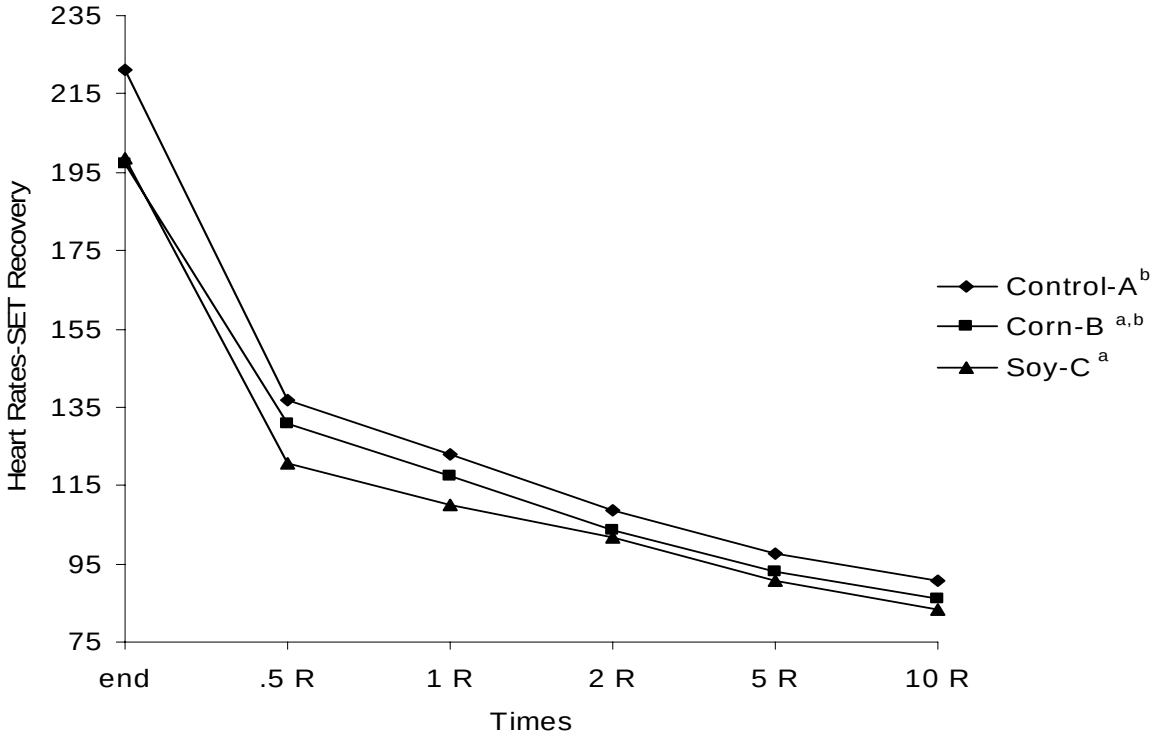


FIGURE 7. EFFECT OF DIET ON MEAN HEART RATES FOR RECOVERY PORTION OF SET

[a,b]Groups lacking common superscripts differ (P<.05).

rates when horses were fed the fat-supplemented diets that began at the end of exercise of the SET (Figure 6).

Cortisol. Plasma cortisol concentrations during the SET rose due to the onset of exercise and remained significantly higher (P<.05) than baseline during the SET from mid-point, end of exercise to 60 R and returned back to baseline by 120 R (Figure 8). As evidenced by the data, peak cortisol concentrations occurred at 15 R and were lowest when horses were in their stalls. There was no difference found in weekly plasma cortisol concentrations due to diet; however, there was an effect of diet on cortisol concentrations during the SET (P<.05). Horses fed soy oil had the lowest serum cortisol concentrations, while horses fed the control diet had the highest values.

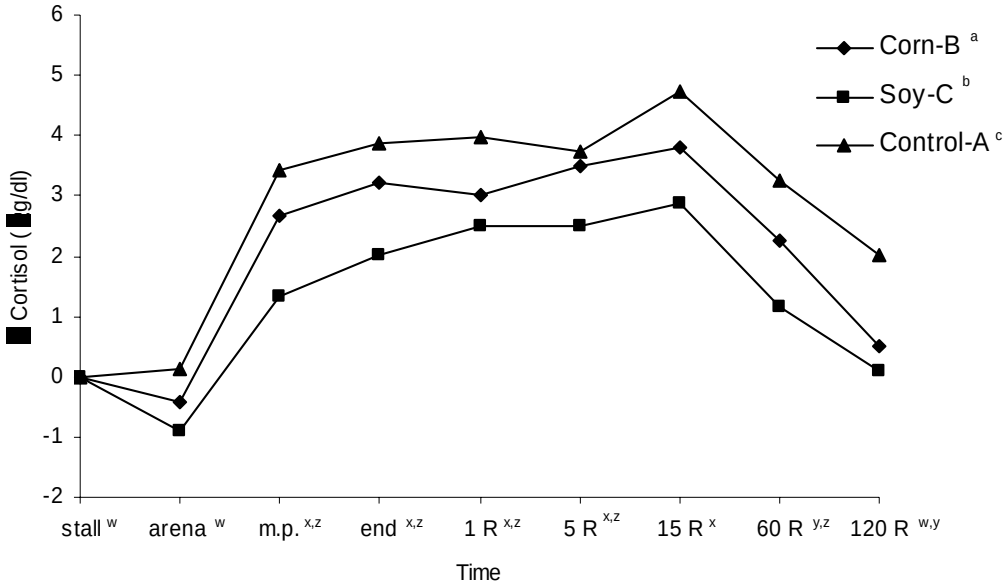


FIGURE 8. EFFECT OF DIET ON PLASMA CORTISOL CONCENTRATIONS DURING SET

[a,b,c]Groups lacking common superscripts differ (P<.05).
[w,x,y,z]Days lacking common superscripts differ (P<.05).

Cholesterol. Serum cholesterol concentrations following 28 days of adaptation to diets are shown in Table 8. Feeding the soy oil diet did not result in significant increases in serum cholesterol concentrations compared to feeding the control diet. However, feeding the corn oil diet resulted in significant increases in serum cholesterol concentrations compared to both the control and soy oil diet.

TABLE 8. EFFECTS OF DIET ON SERUM CHOLESTEROL CONCENTRATIONS AT d 28 (mg/dl)

| Diet | Mean | S.D. |
|---|---|---|
| Control-A[a] | 59.33 | 1.53 |
| Corn-B[b] | 91.00 | 12.73 |
| Soy-C[a] | 67.67 | 2.31 |

[a,b]Groups lacking common superscripts differ (P<.05)

# CHAPTER V

## GENERAL DISCUSSION

A major problem that we are faced with today in the equine industry, is improving the soundness, health and vitality of our young, arena performance horses. It can prove to be tricky; however, attempting this while also maintaining the level of work that you must do to keep your competitive edge in the show arena. Nutrition companies are targeting the new "high energy" feeds, most with elevated levels of fat in them, boasting of their "energy density". Previously and even at current, the soundness of our young horses has been sacrificed by the training regimens and/or injuries that these young horses experience. The glory of winning has been at the expense of our young horse's joint and internal health. Currently, some of the most popular techniques for alleviating joint stress, are quite invasive to the joints themselves.

Feeding added fat to exercising horses is now a common practice in the equine industry. The question now begs, when feeding fat: Which type of fat is the best to feed? There have been studies done showing the benefits of feeding diets rich in n-3 PUFA's to other species, including: lowered heart rates, lowered joint inflammation and increased immune response. In this study, along with a companion study on the same set of horses that looked at the n-3 effects on joint inflammation, we decided to look at the efficacy of administering a diet rich in n-3 PUFA's on the exercising horse's heart rates, plasma cortisol, plasma ACTH and cholesterol concentrations.

Now that we know the benefits of fat-added diets to the exercising horse, including elevated dietary sources of digestible energy, we need to find the fat that is most beneficial to our exercising horses. The most commonly used corn and vegetable

oils, contain n-6:n-3 ratios of 58:1 and higher. The n-3 rich oils, such as that found in fish oil, contains ratios as low as 5:1, n-6:n-3. Omega-3 rich diets have shown to have many benefits in the exercising horse including: lowered inflammatory response, increased arterial function, lowered heart rates and increased immune response. Now that we have seen the benefits that these n-3 rich diets have in our horses, the next logical step would be to research a palatable source of fat with lower n-6:n-3 ratios.

# CHAPTER VI

## SUMMARY AND CONCLUSIONS

In a Latin square experiment, nine three and four-year old American Quarter and Paint horses were blocked by age and gender and randomly divided into three groups. Each group was fed a diet with differing ratios of n-6:n-3 polyunsaturated fatty acids. Diet A consisted of a non-fat supplemented diet that contained an 18.5:1 ratio of n-6:n-3 polyunsaturated fatty acids. Diet B consisted of a corn oil supplemented control that contained a 44.5:1 ratio of polyunsaturated fatty acids. Diet C consisted of an experimental diet supplemented with soybean oil. The soybean oil contained a 9.5:1 ratio of n-6:n-3 polyunsaturated fatty acids, and when added to the concentrate the diet contained a 10.3:1 ratio of n-6:n-3 polyunsaturated fatty acids. Following a 21-d backgrounding period to standardize condition and ability, the horses began a 28-d training and diet adaptation period. During the adaptation period, the horses were ridden in a training protocol designed to mimic the training process of reined cowhorses. The horses were ridden in an aerobic training regimen three days a week. The horses were also ridden at a level to achieve anaerobiosis three times a week while running reining patterns and working a mechanical “cow”. On d 29 the horses were worked on a standardized exercise test to mimic the stresses placed on a young horse during reined cowhorse futurity. Fasting blood plasma samples were analyzed on d 0 and d 32 for polyunsaturated fatty acid profiles. Blood plasma samples taken weekly and during the SET were analyzed by radioimmunoassay for cortistol and ACTH. Blood plasma samples were sent to the Texas Veterinary Medical Diagnostic Lab for cholesterol concentration analysis. Heart rates taken during exercise were analyzed for adaptation to

diet and cardiovascular stress. The horses were maintained in tie stalls for a 4-day total collection of feces to determine the digestibility of dry matter and fat in the diets.

On reining and cutting exercise days, heart rates were lower ($P<.05$) in horses fed diets B and C vs. diet A. However, there were no differences ($P>.05$) in heart rates during exercise on reining and cutting days between horses fed the two fat-supplemented diets. Additionally, there were no significant differences in heart rates due to diet during the exercise portion of the SET. This is explained by the observation that all horses reached near maximal heart rates during the SET. Recovery heart rates following the SET from the end of exercise to 60 R, were significantly lowest in horses fed diet C. Maximum heart rates were achieved following the stopping part of the SET. There was also a trend of lower heart rates when horses were fed the fat-supplemented diets that began at the end of exercise of the SET.

Plasma cortisol concentrations during the SET rose due to the onset of exercise and remained significantly higher ($P<.05$) than baseline during the SET from mid-point, end of exercise to 60 R and returned back to baseline by 120 R. As evidenced by the data, peak cortisol concentrations occurred at 15 R and were lowest when horses were in their stalls. There was no difference found in weekly plasma cortisol concentrations due to diet; however, there was an effect of diet on cortisol concentrations during the SET ($P<.05$). Horses fed soy oil had the lowest serum cortisol concentrations, while horses fed the control diet had the highest values.

Feeding diet C did not result in significant decreases in serum cholesterol concentrations compared to feeding the diet A. However, feeding diet B, resulted in significant increases in serum cholesterol concentrations compared to diets A and C.

There was no significant difference (P>.05) in body weight between horses consuming the three diets. When compared to consuming diet B and diet C the horses fed diet A required significantly more (P<.05) concentrate to maintain constant body weight. However, there were no differences (P>.05) in concentrate intakes between horses fed diets B and C. There was no significant difference in hay intake (P>.05) between horses consuming the three diets. Total feed intake was higher (P<.05) for horses fed diet A than for horses fed diet B. Although numerically higher, total feed intake was not significantly (P>.05) higher for horses fed diet A when compared to horses fed diet C. There was no significant difference (P>.05) in total feed intake for horses fed diets B and C.

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## APPENDIX

TABLE A1. DAILY INTAKES, DRY MATTER DIGESTION, ORGANIC MATTER DIGESTION AND CALCULATED DE

| Horse | Period | Diet | Conc. Intake (kg/day) | Conc. DM % | Conc. DM Intake (kg/day) | Hay Intake (kg/day) | Hay DM % | Hay DM Intake (kg/day) | Total DM Intake (kg/day) | Fecal (kg/day) | Fecal DM % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1A** | 1 | B | 5.0757 | 88.7736 | 4.5059 | 4.2177 | 90.5802 | 3.8204 | 8.3263 | 11.7404 | 27.1075 |
| **2A** | 1 | B | 4.9758 | 88.7736 | 4.4172 | 3.1598 | 90.5802 | 2.8622 | 7.2794 | 10.6054 | 29.7180 |
| **3A** | 1 | B | 4.8124 | 88.7736 | 4.2721 | 3.2052 | 90.5802 | 2.9033 | 7.1755 | 9.6430 | 34.3445 |
| **1B** | 1 | C | 5.3436 | 88.7736 | 4.7437 | 4.5309 | 90.5802 | 4.1041 | 8.8478 | 10.0470 | 30.4837 |
| **2B** | 1 | C | 5.7840 | 88.7736 | 5.1346 | 3.8091 | 90.5802 | 3.4503 | 8.5849 | 9.8473 | 30.7675 |
| **3B** | 1 | C | 6.1653 | 88.7736 | 5.4732 | 4.8987 | 90.5802 | 4.4372 | 9.9104 | 16.8343 | 28.9423 |
| **1C** | 1 | A | 6.2289 | 89.8139 | 5.5944 | 4.4583 | 90.5802 | 4.0383 | 9.6327 | 12.4124 | 32.6745 |
| **2C** | 1 | A | 6.0382 | 89.8139 | 5.4231 | 4.3039 | 90.5802 | 3.8985 | 9.3216 | 13.0661 | 34.2562 |
| **3C** | 1 | A | 5.5206 | 89.8139 | 4.9583 | 3.7001 | 90.5802 | 3.3516 | 8.3099 | 13.5474 | 28.2228 |
| **1A** | 2 | C | 4.6671 | 86.8145 | 4.0514 | 4.4310 | 90.3080 | 4.0016 | 8.0533 | 9.6430 | 26.3703 |
| **2A** | 2 | C | 3.9770 | 86.8145 | 3.4526 | 4.1132 | 90.3080 | 3.7146 | 7.1672 | 9.4114 | 26.2086 |
| **3A** | 2 | C | 4.7216 | 86.8145 | 4.0990 | 3.4459 | 90.3080 | 3.1119 | 7.2109 | 7.6681 | 28.8408 |
| **1B** | 2 | A | 6.3651 | 88.5466 | 5.6361 | 5.2619 | 90.3080 | 4.7519 | 10.3879 | 11.6769 | 32.2527 |
| **2B** | 2 | A | 5.6296 | 88.5466 | 4.9848 | 4.6671 | 90.3080 | 4.2148 | 9.1996 | 8.3899 | 32.7158 |
| **3B** | 2 | A | 6.3651 | 88.5466 | 5.6361 | 4.8169 | 90.3080 | 4.3501 | 9.9861 | 16.0761 | 24.8533 |
| **2C** | 2 | B | 4.1859 | 86.8145 | 3.6340 | 4.3720 | 90.3080 | 3.9483 | 7.5822 | 9.2344 | 32.5856 |
| **3C** | 2 | B | 4.7125 | 86.8145 | 4.0912 | 3.8681 | 90.3080 | 3.4932 | 7.5843 | 10.3921 | 29.8543 |
| **1A** | 3 | A | 5.2210 | 93.5835 | 4.8860 | 3.4504 | 91.1474 | 3.1449 | 8.0309 | 9.9609 | 25.5135 |
| **2A** | 3 | A | 4.8578 | 93.5835 | 4.5461 | 3.2597 | 91.1474 | 2.9712 | 7.5172 | 7.6590 | 27.747 |
| **1B** | 3 | B | 4.4401 | 89.9981 | 3.9960 | 4.2313 | 91.1474 | 3.8567 | 7.8517 | 8.7123 | 29.4385 |
| **2B** | 3 | B | 4.4310 | 89.9981 | 3.9879 | 4.3448 | 91.1474 | 3.9602 | 7.9480 | 8.5715 | 27.8487 |
| **3B** | 3 | B | 4.7034 | 89.9981 | 4.2330 | 4.9032 | 91.1474 | 4.4691 | 8.7021 | 14.7505 | 28.5481 |
| **3C** | 3 | C | 4.8760 | 89.9981 | 4.3883 | 3.5094 | 91.1474 | 3.1987 | 7.5870 | 10.7689 | 27.1947 |

TABLE A1. (CONTINUED).

| Horse | Period | Diet | Fecal DM (kg/d) | Digested DM (kg/d) | Digested DM (% int) | Conc OM Intake (kg/d) | Hay OM Intake (kg/d) | Total OM Intake (kg/d) | Fecal OM (kg/d) | Digested OM (kg/d) | Digested OM (% int) | Calculated DE (Mcal) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1A** | 1 | B | 3.1825 | 5.1437 | 61.7771 | 4.2930 | 3.6028 | 7.8958 | 2.7359 | 5.1599 | 88.03 | 23.7356 |
| **2A** | 1 | B | 3.1517 | 4.1277 | 56.7036 | 4.2085 | 2.6992 | 6.9077 | 2.8148 | 4.0929 | 78.39 | 18.8273 |
| **3A** | 1 | B | 3.3118 | 3.8636 | 53.8450 | 4.0703 | 2.7380 | 6.8083 | 2.7671 | 4.0411 | 74.15 | 18.5893 |
| **1B** | 1 | C | 3.0627 | 5.7851 | 65.384 | 4.5195 | 3.8704 | 8.3900 | 2.5880 | 5.8020 | 87.12 | 26.6893 |
| **2B** | 1 | C | 3.0298 | 5.5551 | 64.7082 | 4.8920 | 3.2538 | 8.1458 | 2.3665 | 5.7793 | 85.90 | 26.5848 |
| **3B** | 1 | C | 4.8722 | 5.0382 | 50.8370 | 5.2146 | 4.1846 | 9.3991 | 4.1345 | 5.2646 | 78.72 | 24.2174 |
| **1C** | 1 | A | 4.0557 | 5.5770 | 57.8964 | 5.3526 | 3.8084 | 9.1609 | 3.5565 | 5.6044 | 32.29 | 23.5385 |
| **2C** | 1 | A | 4.0557 | 5.5770 | 57.8964 | 5.3526 | 3.8084 | 9.1609 | 3.5565 | 5.6044 | 32.29 | 23.5385 |
| **3C** | 1 | A | 3.8234 | 4.4864 | 53.9890 | 4.7440 | 3.1607 | 7.9047 | 3.2287 | 4.6760 | 50.30 | 19.6391 |
| **1A** | 1 | C | 2.5429 | 5.5104 | 68.4244 | 3.8295 | 3.8051 | 7.6345 | 2.1832 | 5.4513 | 84.31 | 25.0759 |
| **2A** | 2 | C | 2.4666 | 4.7006 | 65.5850 | 3.2632 | 3.5322 | 6.7954 | 2.1218 | 4.6736 | 79.05 | 21.4987 |
| **3A** | 2 | C | 2.2115 | 4.9994 | 69.3308 | 3.8742 | 2.9591 | 6.8332 | 1.9089 | 4.9243 | 80.28 | 22.6517 |
| **1B** | 2 | A | 3.7661 | 6.6218 | 63.7453 | 5.4112 | 4.5185 | 9.9298 | 3.1662 | 6.7635 | 67.68 | 28.4067 |
| **2B** | 2 | A | 2.7448 | 6.4548 | 70.1636 | 4.7860 | 4.0078 | 8.7938 | 2.4752 | 6.3186 | 77.55 | 26.5381 |
| **3B** | 2 | A | 3.9955 | 5.9907 | 59.9900 | 5.4112 | 4.1364 | 9.5477 | 3.6196 | 5.9280 | 52.82 | 24.8978 |
| **2C** | 2 | B | 3.0091 | 4.5732 | 60.3141 | 3.4346 | 3.7544 | 7.1890 | 2.5750 | 4.6140 | 81.72 | 21.2244 |
| **3C** | 2 | B | 3.1025 | 4.4819 | 59.0936 | 3.8667 | 3.3216 | 7.1883 | 2.4967 | 4.6916 | 80.19 | 21.5813 |
| **1A** | 3 | A | 2.5413 | 5.4896 | 68.3556 | 4.6689 | 3.0031 | 7.6719 | 2.2519 | 5.4201 | 67.40 | 22.7644 |
| **2A** | 3 | A | 2.1251 | 5.3921 | 71.7298 | 4.3441 | 2.8371 | 7.1812 | 1.8124 | 5.3688 | 55.80 | 22.5489 |
| **1B** | 3 | B | 2.5648 | 5.2880 | 67.3392 | 3.7586 | 3.6827 | 7.4413 | 2.1975 | 5.2439 | 88.07 | 24.1218 |
| **2B** | 3 | B | 2.3871 | 5.5609 | 69.9665 | 3.7509 | 3.7815 | 7.5324 | 2.0791 | 5.4534 | 81.21 | 25.0856 |
| **3B** | 3 | B | 4.2110 | 4.4912 | 51.6099 | 3.9815 | 4.2675 | 8.2491 | 3.7677 | 4.4813 | 69.85 | 20.6141 |
| **3C** | 3 | C | 2.9286 | 4.6585 | 61.4003 | 4.1276 | 3.0544 | 7.1820 | 2.5927 | 4.5893 | 76.18 | 21.1108 |

TABLE A2. FAT DIGESTION

| Horse | Period | Diet | Hay % Fat | Conc % Fat | Hay Fat (kg/day) | Conc Fat (kg/day) | Oil Int. (kg/day) | Total Fat Int (kg/day) | Fecal Extract (kg/day) | Fecal Fat % | Total Dig Fat % Int |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1A | 1 | B | 2.39 | 3.06 | 0.09 | 0.14 | 0.56 | 0.7892 | 3.18 | 2.97 | 88.03 |
| 2A | 1 | B | 2.39 | 3.06 | 0.07 | 0.14 | 0.55 | 0.7536 | 3.15 | 5.17 | 78.39 |
| 3A | 1 | B | 2.39 | 3.06 | 0.07 | 0.13 | 0.54 | 0.7401 | 3.31 | 5.78 | 74.15 |
| 1B | 1 | C | 2.39 | 3.06 | 0.10 | 0.15 | 0.60 | 0.8431 | 3.06 | 3.55 | 87.15 |
| 2B | 1 | C | 2.39 | 3.06 | 0.08 | 0.16 | 0.64 | 0.8825 | 3.02 | 4.12 | 85.90 |
| 3B | 1 | C | 2.39 | 3.06 | 0.11 | 0.17 | 0.69 | 0.9634 | 4.87 | 4.21 | 78.72 |
| 1C | 1 | A | 2.39 | 3.39 | 0.10 | 0.19 | 0.00 | 0.2860 | 4.06 | 4.77 | 32.29 |
| 2C | 1 | A | 2.39 | 3.39 | 0.09 | 0.18 | 0.00 | 0.2769 | 4.47 | 4.11 | 33.66 |
| 3C | 1 | A | 2.39 | 3.39 | 0.08 | 0.17 | 0.00 | 0.2482 | 3.82 | 3.23 | 50.30 |
| 1A | 2 | C | 2.23 | 2.14 | 0.09 | 0.09 | 0.52 | 0.6959 | 2.54 | 4.30 | 84.31 |
| 2A | 2 | C | 2.23 | 2.14 | 0.08 | 0.07 | 0.44 | 0.5967 | 2.47 | 5.06 | 79.05 |
| 3A | 2 | C | 2.23 | 2.14 | 0.07 | 0.09 | 0.53 | 0.6871 | 2.21 | 6.13 | 80.28 |
| 1B | 2 | A | 2.23 | 2.94 | 0.11 | 0.17 | 0.00 | 0.2718 | 3.77 | 2.33 | 67.68 |
| 2B | 2 | A | 2.23 | 2.94 | 0.09 | 0.15 | 0.00 | 0.2404 | 2.74 | 1.97 | 77.55 |
| 3B | 2 | A | 2.23 | 2.94 | 0.10 | 0.17 | 0.00 | 0.2628 | 4.00 | 3.10 | 52.82 |
| 2C | 2 | B | 2.23 | 2.14 | 0.09 | 0.08 | 0.46 | 0.6257 | 3.01 | 3.80 | 81.72 |
| 3C | 2 | B | 2.23 | 2.14 | 0.08 | 0.09 | 0.52 | 0.6854 | 3.10 | 4.38 | 80.19 |
| 1A | 3 | A | 2.25 | 3.58 | 0.07 | 0.17 | 0.00 | 0.2455 | 2.54 | 3.15 | 67.40 |
| 2A | 3 | A | 2.25 | 3.58 | 0.07 | 0.16 | 0.00 | 0.2294 | 2.13 | 4.76 | 55.80 |
| 1B | 3 | B | 2.25 | 2.96 | 0.09 | 0.12 | 0.49 | 0.6951 | 2.56 | 3.24 | 88.07 |
| 2B | 3 | B | 2.25 | 2.96 | 0.09 | 0.12 | 0.49 | 0.6971 | 2.39 | 5.48 | 81.21 |
| 3B | 3 | B | 2.25 | 2.96 | 0.10 | 0.13 | 0.52 | 0.7458 | 4.21 | 5.34 | 69.85 |
| 3C | 3 | C | 2.25 | 2.96 | 0.07 | 0.13 | 0.54 | 0.7419 | 2.93 | 6.03 | 76.18 |

TABLE A3. FATTY ACID COMPOSITION OF FAT CONTAINED IN CONTROL CONCENTRATE

| **Fatty Acid** | **g/100g of identified fatty acids** |
|---|---|
| **14:0** | 0.20 |
| **16:0** | 23.52 |
| **16:1n-7** | 0.22 |
| **18:0** | 3.09 |
| **18:1n-9** | 36.16 |
| **18:2n-6** | 34.55 |
| **18:3n-3** | 1.27 |
| **20:4n-6** | 0.29 |
| **20:5n-3** | 0.71 |

TABLE A4. FATTY ACID COMPOSITION OF FAT CONTAINED IN EXPERIMENTAL CONCENTRATE

| Fatty Acid | g/100g of identified fatty acids |
|---|---|
| **14:0** | 0.23 |
| **16:0** | 25.87 |
| **16:1n-7** | 0.22 |
| **18:0** | 2.72 |
| **18:1n-9** | 33.04 |
| **18:2n-6** | 35.59 |
| **18:3n-3** | 1.59 |
| **20:4n-6** | 0.39 |
| **20:5n-3** | 0.36 |

TABLE A5. FATTY ACID COMPOSITION OF THE CORN OIL

| Fatty Acid | g/100g of identified fatty acids |
|---|---|
| **14:0** | 0.04 |
| **16:0** | 10.84 |
| **16:1n-7** | 0.10 |
| **18:0** | 2.02 |
| **18:1n-9** | 28.92 |
| **18:2n-6** | 28.92 |
| **18:3n-3** | 0.93 |
| **20:4n-6** | 0.21 |
| **20:5n-3** | 0.04 |

TABLE A6. FATTY ACID COMPOSITION OF THE SOYBEAN OIL

| **Fatty Acid** | **g/100g of identified fatty acids** |
|---|---|
| **14:0** | 0.10 |
| **16:0** | 10.72 |
| **16:1N-7** | 0.09 |
| **18:0** | 4.29 |
| **18:1N-9** | 24.13 |
| **18:2N-6** | 54.15 |
| **18:3N-3** | 5.74 |
| **20:4N-6** | 0.74 |
| **20:5n-3** | 0.03 |

TABLE A7. DAY 0 PLASMA FATTY ACID CONCENTRATIONS (g/100g of identified fatty acids)

| Horse | Period | Diet | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:4 | 20:5 | 22:6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1A** | 0 | | 0.95 | 16.39 | 0.61 | 23.40 | 9.49 | 46.06 | 0.76 | 1.11 | 0.10 | 1.13 |
| **2A** | 0 | | 0.86 | 14.27 | 0.74 | 24.71 | 8.76 | 45.36 | 3.02 | 1.64 | 0.22 | 0.42 |
| **3A** | 0 | | 1.01 | 16.11 | 2.28 | 24.20 | 8.63 | 44.59 | 0.77 | 1.98 | 0.42 | 0.00 |
| **1B** | 0 | | 0.85 | 15.63 | 1.56 | 21.27 | 9.03 | 48.38 | 0.79 | 1.95 | 0.39 | 0.14 |
| **2B** | 0 | | 1.02 | 16.70 | 3.30 | 22.55 | 10.54 | 43.34 | 1.36 | 1.19 | 0.00 | 0.00 |
| **3B** | 0 | | 0.99 | 15.19 | 2.89 | 24.57 | 8.10 | 45.35 | 0.70 | 2.21 | 0.00 | 0.00 |
| **1C** | 0 | | 1.23 | 20.81 | 2.12 | 21.17 | 10.31 | 41.52 | 0.55 | 2.10 | 0.19 | 0.00 |
| **2C** | 0 | | 1.01 | 16.40 | 3.07 | 23.42 | 7.47 | 45.17 | 0.47 | 2.35 | 0.51 | 0.14 |
| **3C** | 0 | | 1.15 | 15.61 | 3.69 | 25.89 | 7.37 | 43.38 | 0.65 | 2.27 | 0.00 | 0.00 |

TABLE A8. DAY 32 PLASMA FATTY ACID CONCENTRATIONS

| Horse | Period | Diet | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:4 | 20:5 | 22:6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1A | 1 | B | 0.76 | 14.81 | 0.32 | 23.49 | 8.20 | 49.41 | 1.19 | 1.51 | 0.29 | 0.00 |
| 2A | 1 | B | 0.76 | 13.96 | 5.33 | 24.48 | 6.94 | 46.89 | 0.71 | 0.93 | 0.00 | 0.00 |
| 3A | 1 | B | 8.87 | 15.27 | 2.12 | 22.85 | 7.77 | 48.19 | 0.45 | 1.86 | 0.55 | 0.06 |
| 1B | 1 | C | 0.97 | 16.93 | 2.42 | 21.23 | 6.87 | 49.21 | 0.52 | 1.60 | 0.25 | 0.00 |
| 2B | 1 | C | 1.03 | 14.43 | 2.56 | 25.32 | 3.68 | 47.13 | 0.74 | 1.68 | 0.43 | 0.30 |
| 3B | 1 | C | 0.69 | 13.29 | 1.96 | 23.09 | 6.59 | 50.32 | 0.76 | 2.25 | 0.63 | 0.15 |
| 1C | 1 | A | 0.97 | 15.61 | 2.26 | 23.46 | 10.39 | 44.09 | 0.89 | 1.84 | 0.37 | 0.12 |
| 2C | 1 | A | 0.98 | 16.15 | 2.71 | 23.01 | 8.08 | 47.14 | 0.75 | 2.08 | 0.09 | 0.00 |
| 3C | 1 | A | 1.11 | 16.33 | 2.86 | 23.10 | 8.53 | 44.32 | 0.88 | 2.21 | 0.55 | 0.11 |
| 1A | 2 | C | 0.63 | 13.46 | 2.42 | 21.91 | 6.78 | 51.51 | 0.68 | 1.88 | 0.59 | 0.13 |
| 2A | 2 | C | 0.47 | 11.51 | 1.70 | 19.89 | 8.97 | 51.53 | 1.40 | 1.48 | 0.63 | 2.40 |
| 3A | 2 | C | 0.79 | 13.95 | 2.11 | 21.42 | 7.37 | 50.31 | 0.59 | 2.92 | 0.51 | 0.03 |
| 1B | 2 | A | 1.02 | 16.92 | 3.98 | 21.90 | 9.46 | 44.51 | 0.42 | 1.80 | 0.00 | 0.00 |
| 2B | 2 | A | 0.92 | 19.18 | 1.38 | 19.60 | 11.02 | 45.66 | 0.77 | 1.33 | 0.06 | 0.09 |
| 3B | 2 | A | 1.02 | 16.25 | 2.51 | 22.57 | 9.29 | 46.65 | 0.60 | 2.11 | 0.00 | 0.00 |
| 2C | 2 | B | 0.67 | 13.33 | 1.64 | 20.40 | 8.54 | 52.02 | 0.43 | 2.00 | 0.92 | 0.05 |
| 3C | 2 | B | 0.63 | 15.15 | 2.00 | 18.18 | 9.72 | 51.41 | 0.28 | 1.10 | 0.58 | 0.92 |
| 1A | 3 | A | 0.75 | 16.31 | 0.86 | 20.59 | 10.59 | 48.88 | 0.57 | 1.46 | 0.00 | 0.00 |
| 2A | 3 | A | 0.83 | 14.17 | 3.26 | 21.86 | 9.23 | 44.99 | 1.37 | 2.58 | 0.00 | 1.72 |
| 1B | 3 | B | 0.66 | 14.47 | 1.80 | 20.59 | 8.30 | 51.84 | 0.23 | 1.67 | 0.45 | 0.00 |
| 2B | 3 | B | 0.59 | 12.74 | 1.45 | 21.29 | 9.09 | 50.29 | 0.43 | 2.70 | 1.19 | 0.23 |
| 3B | 3 | B | 0.60 | 13.92 | 1.64 | 20.55 | 7.94 | 52.84 | 0.47 | 1.86 | 0.17 | 0.00 |
| 3C | 3 | C | 0.56 | 14.60 | 1.97 | 19.12 | 7.70 | 54.01 | 0.94 | 1.07 | 0.03 | 0.000 |

TABLE A9. ANOVA TABLE FOR MEAN WEIGHTS

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 54183.348 | 13545.837 | 0.99 | 0.4371 |
| Period | 2 | 53338.2882 | 26669.1411 | 1.95 | 0.1708 |
| Diet | 2 | 343.746524 | 171.873262 | 0.01 | 0.9875 |
| Residual | 18 | 245817.887 | 13656.5048 | | |
| Total | 22 | 300000.435 | 13636.3834 | | |

TABLE A10. ANOVA TABLE FOR TOTAL DAILY CONCENTRATE INTAKE

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 6.42521909 | 1.60630477 | 9.83 | 0.0002 |
| Period | 2 | 1.690118071 | .845090354 | 5.17 | 0.0168 |
| Diet | 2 | 4.475557183 | 2.37778592 | 14.55 | 0.0002 |
| Residual | 18 | 2.94185159 | .163436199 | | |
| Total | 22 | 9.36707068 | .42577594 | | |

TABLE A11. ANOVA TABLE FOR DAILY HAY INTAKE

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | .634759072 | .158689768 | 0.49 | 0.7432 |
| Period | 2 | .504820389 | .252410195 | 0.78 | 0.4737 |
| Diet | 2 | .100253438 | .050126719 | 0.15 | 0.8578 |
| Residual | 18 | 5.83118385 | .323954659 | | |
| Total | 22 | 6.46594293 | .293906497 | | |

TABLE A12. ANOVA TABLE FOR TOTAL DAILY INTAKE

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 7.74229538 | 1.93557384 | 2.57 | 0.0729 |
| Period | 2 | 1.30876858 | .654384291 | 0.87 | 0.4357 |
| Diet | 2 | 6.1590728 | 3.0795364 | 4.10 | 0.0342 |
| Residual | 18 | 13.534349 | .751908275 | | |
| Total | 22 | 21.2766443 | .967120197 | | |

TABLE A13. ANOVA TABLE FOR DIGESTIBLE DRY MATTER

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 4.15524443 | 1.03881111 | 2.84 | 0.0549 |
| Period | 2 | .795040865 | .397520433 | 1.09 | 0.3585 |
| Diet | 2 | 3.17526649 | 1.58763325 | .34 | 0.0290 |
| Residual | 18 | 6.58494142 | .365830079 | | |
| Total | 22 | 10.7401858 | .488190266 | | |

TABLE A14. ANOVA TABLE FOR DRY MATTER DIGESTIBILITY

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 361.198734 | 90.2996835 | 2.77 | 0.0589 |
| Period | 2 | 312.001823 | 156.000912 | 4.79 | 0.0215 |
| Diet | 2 | 65.9733939 | 32.9866969 | 1.01 | 0.3830 |
| Residual | 18 | 586.251583 | 32.5695324 | | |
| Total | 22 | 947.450317 | 43.0659235 | | |

TABLE A15. ANOVA TABLE FOR DAILY CONCENTRATE INTAKE

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 4.90713867 | 1.22678467 | 2.83 | 0.0554 |
| Period | 2 | .865624148 | .432812074 | 1.00 | 0.3879 |
| Diet | 2 | 3.4981496 | 1.7490748 | 4.04 | 0.0356 |
| Residual | 18 | 7.80137796 | .433409886 | | |
| Total | 22 | 12.7085166 | .577659847 | | |

TABLE A16. ANOVA TABLE FOR ORGANIC MATTER DIGESTIBILITY

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 200.407488 | 50.1018721 | 2.08 | 0.1262 |
| Period | 2 | 157.662583 | 78.8312912 | 3.27 | 0.0615 |
| Diet | 2 | 47.4850856 | 23.7425428 | 0.98 | 0.3929 |
| Residual | 18 | 434.131043 | 24.1183913 | | |
| Total | 22 | 634.538531 | 28.8426605 | | |

TABLE A17. ANOVA TABLE FOR FAT INTAKE

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 1300399.47 | 325099.867 | 98.88 | 0.00000 |
| Period | 2 | 60211.0397 | 30105.5199 | 9.16 | 0.0018 |
| Diet | 2 | 1222167.35 | 611083.673 | 185.87 | 0.0000 |
| Residual | 18 | 59177.9187 | 3287.66215 | | |
| Total | 22 | 1359577 | 61798.9722 | | |

TABLE A18. ANOVA TABLE FOR DIGESTED FAT

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 1152574.79 | 288143.697 | 57.82 | 0.0000 |
| Period | 2 | 25242.7591 | 12621.3795 | 2.53 | 0.1074 |
| Diet | 2 | 1115948.52 | 557974.262 | 111.96 | 0.0000 |
| Residual | 18 | 89707.2726 | 4983.73737 | | |
| Total | 22 | 1242282.06 | 56467.3663 | | |

TABLE A19. ANOVA TABLE FOR PERCENT DIGESTED FAT

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 3929.08133 | 982.270332 | 9.53 | 0.0003 |
| Period | 2 | 324.0573 | 171.02865 | 1.66 | 0.2180 |
| Diet | 2 | 3655.05325 | 1827.52662 | 17.74 | 0.0000 |
| Residual | 18 | 1854.5987 | 103.033261 | | |
| Total | 22 | 5783.68003 | 262.894547 | | |

TABLE A20. ANOVA TABLE FOR MEAN MYRISTIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 0.515500641 | 0.12887516 | 11.43 | 0.0001 |
| Period | 2 | 0.213448155 | 0.106724078 | 9.47 | 0.0015 |
| Diet | 2 | 0.300786443 | 0.150393222 | 13.34 | 0.0003 |
| Residual | 18 | 0.202873276 | 0.11270738 | | |
| Total | 22 | 0.718373917 | 0.03265336 | | |

TABLE A21. ANOVA TABLE FOR MEAN PALMITIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 25.696726 | 6.4241815 | 3.35 | 0.0323 |
| Period | 2 | 2.16399007 | 1.08249503 | 0.56 | 0.5782 |
| Diet | 2 | 23.6781143 | 11.8390572 | 6.18 | 0.0091 |
| Residual | 18 | 34.5002754 | 1.91668197 | | |
| Total | 22 | 60.1970014 | 2.73622734 | | |

TABLE A22. ANOVA TABLE FOR MEAN PALMITOLEIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 2.40467126 | 0.601167815 | 0.53 | 0.7159 |
| Period | 2 | 1.58665321 | 0.793326605 | 0.70 | 0.5105 |
| Diet | 2 | 0.765582749 | 0.382791375 | 0.34 | 0.7184 |
| Residual | 18 | 20.4541891 | 1.13634384 | | |
| Total | 22 | 22.8588603 | 1.03903911 | | |

TABLE A23. ANOVA TABLE FOR MEAN STEARIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 39.2260088 | 9.08650219 | 6.23 | 0.0025 |
| Period | 2 | 39.0862954 | 19.0431477 | 12.09 | 0.0005 |
| Diet | 2 | 1.26996154 | 0.634980771 | 0.40 | 0.6741 |
| Residual | 18 | 28.3549546 | 1.57527526 | | |
| Total | 22 | 67.5809634 | 3.07186197 | | |

TABLE A24. ANOVA TABLE FOR MEAN OLEIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 26.5328996 | 6.63322491 | 10.83 | 0.0001 |
| Period | 2 | 5.99763269 | 2.99881635 | 4.90 | 0.0200 |
| Diet | 2 | 19.7928217 | 9.8641087 | 16.16 | 0.0001 |
| Residual | 18 | 11.024252 | 0.12458446 | | |
| Total | 22 | 37.5571517 | 1.70714326 | | |

TABLE A25. ANOVA TABLE FOR MEAN LINOLEIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 157.791683 | 39.4479208 | 18.32 | 0.0000 |
| Period | 2 | 37.1295838 | 18.5647919 | 28.55 | 0.0024 |
| Diet | 2 | 122.994524 | 61.4972621 | 8.62 | 0.0000 |
| Residual | 18 | 38.7690724 | 2.15383736 | | |
| Total | 22 | 196.560756 | | | |

TABLE A26. ANOVA TABLE FOR MEAN LINOLENIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 0.464514504 | 0.069093835 | 0.22 | 0.9239 |
| Period | 2 | 0.087100096 | 0.029335435 | 0.09 | 0.9114 |
| Diet | 2 | 0.396746688 | 0.124380058 | 0.40 | 0.6790 |
| Residual | 18 | 1.66194639 | 0.314421652 | | |
| Total | 22 | 2.1264609 | 0.269816594 | | |

TABLE A27. ANOVA TABLE FOR MEAN ARACHIDONIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 0.276375339 | 0.069093835 | 0.22 | 0.9239 |
| Period | 2 | 0.05867087 | 0.029335435 | 0.09 | 0.9114 |
| Diet | 2 | 0.248760116 | 0.124380058 | 0.40 | 0.6790 |
| Residual | 18 | 5.65958974 | 0.314421652 | | |
| Total | 22 | 5.93596508 | 0.269816594 | | |

TABLE A28. ANOVA TABLE FOR MEAN EICOSAPENTAENOIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 0.724434087 | 0.181108522 | 2.03 | 0.1331 |
| Period | 2 | 0.070088148 | 0.035049575 | 0.39 | 0.6808 |
| Diet | 2 | 0.685658167 | 0.342824083 | 3.84 | 0.0408 |
| Residual | 18 | 1.60586166 | 0.089214537 | | |
| Total | 22 | 2.33029575 | 0.105922534 | | |

TABLE A29. ANOVA TABLE FOR MEAN DOCOXAHEXAENOIC ACID ON d 32

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 0.880864041 | 0.22021601 | 0.55 | 0.6999 |
| Period | 2 | 0.599048796 | 0.299524398 | 0.75 | 0.4860 |
| Diet | 2 | 0.279354342 | 0.139677171 | 0.35 | 0.7092 |
| Residual | 18 | 7.17670169 | 0.398705649 | | |
| Total | 22 | 8.05756573 | | | |

TABLE A30. ANOVA TABLE FOR PERCENT CHANGE IN MYRISTIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 8015.09479 | 2003.7737 | 3.67 | 0.0236 |
| Period | 2 | 568.92388 | 284.46194 | 0.52 | 0.6029 |
| Diet | 2 | 7824.28418 | 3912.14209 | 7.16 | 0.0052 |
| Residual | 18 | 9836.69189 | 546.482883 | | |
| Total | 22 | 17851.7867 | 811.444849 | | |

TABLE A31. ANOVA TABLE FOR MEAN PERCENT CHANGE IN PALMITIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 2166.72558 | 541.681394 | 2.75 | 0.0605 |
| Period | 2 | 159.136831 | 79.5684157 | 0.40 | 0.6737 |
| Diet | 2 | 1955.70577 | 977.852883 | 4.96 | 0.0192 |
| Residual | 18 | 3547.693 | 197.094055 | | |
| Total | 22 | 5714.41857 | 259.746299 | | |

TABLE A32. ANOVA TABLE FOR CHANGE IN PALMITOLEIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 29.854485 | 182.463621 | 2.52 | 0.0772 |
| Period | 2 | 683.569959 | 341.78979 | 4.72 | 0.0224 |
| Diet | 2 | 117.957247 | 58.9756235 | 0.82 | 0.4582 |
| Residual | 18 | 1302.29135 | 72.3495195 | | |
| Total | 22 | 2032.14584 | 92.3702653 | | |

TABLE A33. ANOVA TABLE FOR CHANGE IN STEARIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 729.854485 | 182.463621 | 2.52 | 0.0772 |
| Period | 2 | 683.569959 | 341.784979 | 4.72 | 0.0224 |
| Diet | 2 | 117.957247 | 58.9786235 | 0.82 | 0.4582 |
| Residual | 18 | 1302.29135 | 72.3495195 | | |
| Total | 22 | 2032.14585 | 92.3702653 | | |

TABLE A34. ANOVA TABLE FOR CHANGE IN OLEIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 13189.4136 | 3297.35341 | 14.26 | 0.0000 |
| Period | 2 | 4572.18041 | 2286.09021 | 9.89 | 0.0013 |
| Diet | 2 | 8303.47436 | 4151.73718 | 17.95 | 0.0000 |
| Residual | 18 | 4162.34348 | 231.241304 | | |
| Total | 22 | 17351.7571 | 788.716233 | | |

TABLE A35. ANOVA TABLE FOR CHANGE IN LINOLEIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 876.613174 | 219.153294 | 7.14 | 0.0012 |
| Period | 2 | 22.7785905 | 11.3892953 | 0.37 | 0.6953 |
| Diet | 2 | 837.298748 | 418.649371 | 13.63 | 0.0002 |
| Residual | 18 | 552.575356 | 30.708742 | | |
| Total | 22 | 1429.37053 | 64.7913877 | | |

TABLE A36. ANOVA TABLE FOR CHANGE IN LINOLENIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 20021.987 | 10010.9935 | 2.41 | 0.1157 |
| Period | 2 | | | | |
| Diet | 2 | 20021.987 | 10010.9935 | 2.41 | 0.1157 |
| Residual | 18 | 83.199.2783 | 4159.96391 | | |
| Total | 22 | 103221.265 | 4691.87569 | | |

TABLE A37. ANOVA TABLE FOR CHANGE IN ARACHIDONIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 5330.90304 | 1332.72576 | 0.91 | 0.4766 |
| Period | 2 | 2575.23071 | 1287.61536 | 0.88 | 0.4303 |
| Diet | 2 | 3559.14542 | 1779.57271 | 1.22 | |
| Residual | 18 | 26224.3975 | 1456.91097 | | |
| Total | 22 | 31555.3005 | 1434.33184 | | |

TABLE A38. ANOVA TABLE FOR CHANGE IN EICOSAPENTAENOIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 9608.74204 | 2402.18551 | 1.53 | 0.2368 |
| Period | 2 | 276.246996 | 138.123498 | 0.09 | 0.9164 |
| Diet | 2 | 9356.2333 | 4678.11665 | 2.97 | 0.0767 |
| Residual | 18 | 28332.5838 | 1574.03243 | | |
| Total | 22 | 37941.3259 | 1724.60572 | | |

TABLE A39. ANOVA TABLE FOR CHANGE IN DOCOXEHEXAENOIC ACID

| Source | df | Partial SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4 | 16397.4335 | 4099.35836 | 1.35 | 0.2911 |
| Period | 2 | 9488.79336 | 4744.39668 | 1.56 | 0.2374 |
| Diet | 2 | 5544.97981 | 2772.48991 | 0.91 | 0.4198 |
| Residual | 18 | 54772.0721 | 3042.89289 | | |
| Total | 22 | 71169.5055 | 3234.97752 | | |

# VITA

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|---|---|
| NAME: | Alicia Dawn Howard |
| EDUCATIONAL BACKGROUND: | B.S., Animal Science (2000)<br>Texas A&M University<br>College Station, TX |
| PERMANENT ADDRESS: | 2251 CR 127<br>Georgetown, TX 78626 |
| PROFESSIONAL EXPERIENCE: | Graduate Extension Assistant,<br>Department of Animal Science,<br>Texas A&M University, College Station<br>Research: Animal Science<br>Teaching: Animal Science<br>Horse Judging, Horse Training |