EFFECT OF N-3 PUFAS ON MARKERS OF INFLAMMATION IN ARTHRITIC HORSES

A Thesis

by

DENISE RAE MANHART

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 2007

Major Subject: Animal Science

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Approved by:

Co-Chairs of Committee,	Brett Scott
	Pete Gibbs
Committee Members,	Josie Coverdale
	David Hood
Head of Department	Gary Acuff

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ABSTRACT

Effect of n-3 PUFAs on Markers of Inflammation in Arthritic

Horses.

(August 2007)

Denise Rae Manhart, B.S., Oregon State University

Co-Chairs of Advisory Committee: Dr. Brett Scott
Dr. Pete Gibbs

Sixteen horses with at least one arthritic joint were randomly divided into two groups. The control group (n=8) was fed a control ration at 1% BW in grain. The treatment group (n=8) was fed an isocaloric diet similar to the control diet with additional n-3 polyunsaturated fatty acids (PUFAs) in the form of two pelleted supplements. Coastal hay was fed free choice, and both groups consumed their respective diet for 90 days.

On d 0, 30, 60, and 90 synovial fluid was collected from one arthritic joint on each horse, and blood samples were collected every 15 days. Synovial fluid was analyzed for Tumor Necrosis Factor-α, Interleukin-1, and white blood cell concentration, and plasma was analyzed for fibrinogen and Prostaglandin E_2. Force plate analysis was used to determine changes in weight distribution throughout the trial.

Fatty acid analysis revealed the main n3 supplied by the supplements was docosahexaenoic acid (C22:6n3). Treatment horses consumed 9.3 g docosahexaenoic acid daily, while control horses consumed only 0.42 g daily. A reduction in concentrate intake also allowed treatment horses to consume 25.45 g less of linoleic acid (C18:2n6)

per day. Excluding hay, the n6:n3 ratio of the treatment diet was 5:1 compared to the control diet with a ratio of 11:1. Analysis of plasma fatty acid profiles revealed treatment horses experienced an increase in plasma docosahexaenoic acid, along with a decrease in linoleic acid (C18:2n6). Total plasma n6:n3 ratio of treatment horses was 23:1, as opposed to 27:1 in the control horses.

Treatment horses had significantly lower synovial fluid white blood cell concentration and plasma Prostaglandin E_2 ($P < 0.05$). A trend towards decreased fibrinogen ($P = 0.076$) was also seen in the treatment horses. Synovial fluid TNF-α and IL-1 concentrations were not obtained due to problems with the assay kits or procedures. Force plate data from seven horses was analyzed. No significant increase in weight placed on arthritic limbs ($P = 0.12$) was seen.

This data provides further evidence that a decrease in the n6:n3 ratio of the diet and plasma can lead to a decrease in the production of inflammatory compounds in arthritic joints.

DEDICATION

For Belle

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CHAPTER I

INTRODUCTION

Arthritis is the most significant cause of lameness in horses (Todhunter et al., 1990) and can lead to the early retirement of otherwise healthy animals. Arthritic joints result from previous damage to the joint, wear and tear due to exercise, as well as the natural aging process. As young animals, cartilage formation is at its highest rate and young horses can easily repair and replace damaged joint tissue. However, as horses age, the abilities of their joints to repair themselves becomes limited. Eventually cartilage metabolism becomes greater than anabolism; therefore, even small amounts of damage can escalate to chronic lameness (Brama et al., 2000). Many current treatments for equine arthritis focus only on relieving pain and do not attempt to prevent further joint degradation. In addition, some common pain-relieving treatments, such as non-steroidal anti-inflammatory drugs (NSAIDS), can lead to other health problems when used continuously. Research has shown that n3 polyunsaturated fatty acids (PUFAs) may safely decrease the inflammation and pain caused by arthritis, as well as possibly slowing the degradation of the joint (Cleland et al., 1988; Curtis et al., 2000; Munsterman et al., 2005).

Once injury or degradation of cartilage occurs in a joint, inflammation is caused by mediators in an attempt to stop the damage. Numerous non-specific compounds, such as mast cell granules, are released to alert the body of the insult, thus initiating a cascade of inflammatory events starting with the infiltration of polymorphonuclear

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This thesis follows the format of the Journal of Animal Science.

leukocytes and monocytes to the site of injury. The ultimate result is the production and release of inflammatory cytokines and eicosanoids from local cells. Initially, this process is designed to limit use of the joint to promote healing, yet with continuous exercise and damage, excessive amounts of eicosanoids are released resulting in chronic inflammation, pain, and further degradation of the cartilage by elevated metalloproteinases (Palmer and Bertone, 1994**)**.

Examples of common inflammatory cytokines include tumor necrosis factor- α (TNF-α), interleukin-1 (IL-1), and interleukin6 (IL-6). These molecules have paracrine and autocrine properties which can further enhance their production and release. Cytokine release leads to the production of inflammatory eicosanoids such as Prostaglandin E_2 (PGE_2) and Leukotrienes, which are synthesized through metabolism of long chain PUFAs. Arachidonic acid (AA, C20:4) is a common dietary n6 fatty acid which, through the cyclooxygenase and lipooxygenase pathways, is a precursor for PGE_2 production, along with other inflammatory products such as Leukotriene B_4 (LTB_4) (Calder, 2002). Therefore, it is hypothesized that increased levels of n6 fatty acids in the plasma may lead to an increased production of inflammatory eicosanoids, enhancing the inflammatory response. In equine, as well as human diets, n6 fatty acids are found in high concentrations compared to n3s. Linoleic acid (LA, C18:2n6), found in corn and corn oil, safflower oil, soybean oil, and AA, found in meat and animal products, are the most common n6 PUFAs. Therefore the common practice of adding corn oil as a fat supplement for horses may actually be increasing the animal's inflammatory potential.

Omega-3 fatty acids, such as eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) compete with n6 fatty acids for storage in the cell membrane, as well as during metabolism. When they are metabolized via the cyclooxygenase and lipooxygenase pathways, n3 PUFAs result in either less potent eicosanoids, or anti-inflammatory eicosanoids such as Prostaglandin E_1, Thromboxane A_3 and Leukotriene B_5 (Calder, 2002). Because of this difference in metabolism it is believed that the addition of n3 PUFAs to the diet may decrease inflammation and the potential pain it causes.

CHAPTER II

REVIEW OF LITERATURE

Joint Health

The degeneration of articular cartilage is the end result of osteoarthritis. The main components of cartilage are collagen and proteoglycan, which are both present in many forms. Collagen content is divided into various types (II, V, V, IX, XI), with type II occurring predominantly (Vachon et al., 1990). Proteoglycan, formed in large aggregates in collagen, consists of numerous types in equine joints with each type having different properties and found in differing amounts depending on the horse's age. In general, proteoglycans provide high osmotic pressure in the joint and allow the cartilage to retain large amounts of water, cushioning the impact from flexion and load bearing (Maroudas et al., 1986). Though damage to proteoglycans in the joint can be reversed, the collagen network has a very limited ability to repair itself, especially that of adult animals. Therefore, joint damage caused by injury or age could be defined as permanent.

Insult or injury to a joint results in inflammation to prevent further damage and promote rest and healing in the joint capsule. Similar to the process of acute inflammation in other body tissues, this process leads to the release of IL-1, produced by the synovial lining cells and macrophages. This acute phase activator induces the production of phospholipase A_2 which releases fatty acids from cellular phospholipids. These fatty acids are then metabolized through the cyclooxygenase and lipooxygenase pathways resulting in the production of eicosanoids, such as inflammatory PGE_2 (Pujol and Loyou, 1987). Interleukin-1 can also lead to the synthesis of metallproteoglycanase

and collagenase in chondrocytes. These enzymes are known to degrade the collage matrix (Mizel, 1989). Though the inflammatory process is designed to promote healing of the tissue, continuous use and damage results in the constant production of IL-1 and the resultant joint-damaging compounds.

As noted previously, the precursor to inflammatory eicosanoids such as PGE_2 is arachidonic acid (AA). As the AA (C20:4) content of membranes increases, the ability of cells such as chondrocytes and synovial cells to produce inflammatory products is enhanced. With increasing severity of histological damage to human joints, an increase in lipid accumulation in the articular cartilage was noted by Lippiello et al. (1991). Specifically, AA levels increased 393% and 1,150% in mild and severe cases of joint injury, respectively. This suggests free fatty acids are involved in the initial response during the development of joint disease. Biochemically, n3 PUFAs are known to compete with n6s for storage in the cell, release from the cell membrane, and metabolism. The concentration of n3s in the cell membrane is dependent upon n3 consumption by the animal. An increase in intake will lead to increased n3s and decreased n6s in the cell. A subsequent increase in the rate of release from the cell membrane by Phospholipase A_2 and further n3 metabolism will then occur. In addition, products from the metabolism of n3s, such as Prostaglandin E_3, are less inflammatory than those from n6s. Therefore, a higher intake of n3s, leading to increased levels of n3s in the membrane and a resultant decline in membrane n6s, can decrease the ability of cells to produce inflammatory products after insult to a joint (Calder, 2002). It has also been suggested that n3s can decrease production of inflammatory cytokines at the level of gene expression by way of signal transduction (Calder, 2002).

Various treatments are available for the management of osteoarthritis in horses. Non-steroidal anti-inflammatory drugs (NSAIDS) such as phenylbutazone are utilized for pain management. However, continuous usage of NSAIDS at high doses can lead to other health problems such as stomach ulcers (MacAllister et al., 1993). Recently, oral supplements containing glucosamine and/or chondroitin sulfate have become very popular; however, research has yet to verify their efficacy. Both compounds are required for the repair and maintenance of joints, and ideally consuming additional amounts of one or both compounds will provide an excess of resources required to maintain a healthy joint. Research has focused on how effective these compounds are in repairing joints when consumed orally. The bioavailability of chondroitin sulfate in equines has been estimated at 13.2% of the amount ingested (Conte et al., 1991). This percentage is then available in the bloodstream for incorporation into tissues, such as cartilage. However, even if available in the plasma, both chondroitin sulfate and glucosamine can be filtered out of the blood after one pass through the liver further decreasing their usefulness in the body.

Numerous studies investigating the effects of oral supplements have found beneficial results, however, some researchers question the validity of these results. McAlindon et al. (2000) performed a meta-analysis and quality assessment of 15 clinical trials testing the efficacy of oral chondroitin sulfate and glucosamine supplements as treatments for arthritis in horses. Most individual trials demonstrated moderate positive effects; however, they observed major deficiencies in randomization, blinding and completion rates in trials. Therefore, these positive results may not be trustworthy and alternative treatments need to be explored.

Omega-3 Fatty Acids

Numerous in vivo and in vitro studies have investigated the anti-inflammatory effects of n3 PUFAs in horses, mice, and humans. Curtis et al. (2000) used bovine cartilage in vitro to study the effect of increasing levels of n3s in the membrane of IL-1 stimulated chondrocytes. Higher levels of n3s in the cell membranes resulted in a dose-dependent reduction in the expression of TNF-α and IL-1, as well as a decrease in the expression of proteoglycan-degrading enzymes such as aggrecanase. Stride length was used by Woodward et al. (2005) to measure lameness in arthritic horses fed supplemental EPA (C20:5) and DHA (C22:6). A trend towards greater trot stride length was observed in horses consuming 15 g of supplemental EPA and DHA per day, indicating a reduction in lameness. These results provide evidence that n3 PUFA supplementation may be beneficial to the arthritic joint.

Research has also been conducted to evaluate n3 supplementation in the formation of inflammatory markers in other animals. Eicosanoid production was stimulated in healthy mice consuming varying levels of n6 and n3 fatty acids (Li et al., 1994). Pooled eicosanoid production was lower in mice consuming additional EPA. It was also noted that mice consuming supplemental EPA (C20:5n3) and AA (C20:4n6) had similar eicosanoid production as mice consuming only AA. Due to these results the authors illustrated the importance of feeding only one type of fatty acid per treatment group due to a suppressive effect of AA on EPA metabolism. Song et al. (2003) observed a similar response when feeding Wistar rats ethyl-EPA and using IL-1 to induce stress and inflammation. Consumption of ethyl-EPA reduced the elevated PGE_2 secretion and increased secretion of the anti-inflammatory cytokine Interleukin-10.

Treatment with EPA also significantly blocked the elevation of corticosterone released in response to anxiety. Therefore, elevated intakes of EPA versus AA may be effective at decreasing the production of inflammatory markers such as eicosanoids and cytokines.

A large amount of n3 research has also been conducted in humans with rheumatoid arthritis (RA). In one study, patients with RA consumed 3.2 g EPA (C20:5) and 2.0 g DHA (C22:6) daily compared to the control group which consumed 1g olive oil daily, which is believed to contain neutral fatty acids, for 12 weeks. Tender joint score and grip strength were improved in the treatment group, along with a decreased production of LTB_4 by isolated neutrophils (Cleland et al., 1988). A study conducted by Kremer et al. (1990) resulted in a 54.7% decrease in macrophage IL-1 and a 20.0% decrease in neutrophil leukotriene B_4 (LTB_4) in a group of RA patients receiving 54 mg/kg of EPA and 36 mg/kg of DHA for 24 weeks. Patients also experienced a significant reduction in tender and swollen joints. Lau et al. (1993) reported similar results in patients with RA. Patients consuming fish oil, which naturally contains high amounts of EPA and DHA, were able to significantly reduce their level of RA medication (NSAIDS) without an increase in pain or other symptoms.

In addition, Adam et al. (2003) noted patients that consumed an anti-inflammatory diet containing less than 90 mg/day of AA showed significantly lower formation of LTB_4 and prostaglandin metabolites, compared to patients consuming a typical diet. In addition, by simply reducing the intake of AA (C20:4n6), patients had a higher enrichment of EPA (C20:5n3) in erythrocyte lipids. When fish oil was included in the low AA diet, the anti-inflammatory benefits indicated above were even greater.

These studies provide evidence that decreasing consumption of n6s alone can reduce problems associated with joint disease.

Measuring Joint Health

It is difficult to objectively determine the presence and extent of joint disease in animals. It is suggested that levels of inflammatory cytokines and eicosanoids in blood and synovial fluid could be used as markers of arthritis. Though blood samples are easier to obtain, concentrations of these compounds in synovial fluid have been shown to be more indicative of joint disease. Serum or plasma concentrations reflect the condition of the entire body, rather than just diseased joints. Many joint markers may also be eliminated by the time they reach the vascular system. Eicosanoid and cytokine concentrations are also much higher in the synovial fluid and therefore should be easier to detect and monitor (Lohmander et al., 1992). Synovial fluid white blood cell (WBC) count is commonly used to identify joint problems, and the concentration in normal equine joints has been reported as 167 $\pm$ 21 cells/ml and 76 $\pm$ 99 cells/ml, respectively (Van Pelt, 1974; Persson, 1971). However, it is also noted in traumatic arthritis and osteoarthritis that the cell count may vary tremendously (McIlwraith et al., 2001).

Little research has been done to determine a normal concentration range for many eicosanoids or cytokines in equine plasma and synovial fluid. However, Bertone et al. (2001) conducted a study to verify the sensitivities of many compounds and their ability to predict joint disease and changes in joint health. Synovial fluid prostaglandin E_2 and IL-6 were found to be excellent predictors of joint disease in horses, while TNF-α and IL-1 were no more effective than a white blood cell count. In addition, Kirker-Head et al. (2000) found PGE_2 concentrations were increased in equine osteoarthritic

joints compared with normal joints. However, Bertone et al. (2001) also noted PGE_2 concentration was highly variable among horses. In addition, though synovial fluid PGE_2 is a very sensitive marker, and therefore able to detect minor problems, the magnitude of its production and release does not appear to be well correlated to the degree of insult. A minor insult can result in PGE_2 concentrations similar to those after a major insult in the same horse. Synovial fluid IL-1 and TNF-α are not as sensitive to small insults; however, they are less variable between horses and concentrations are more strongly correlated to the magnitude of insult (Bertone et al., 2001). This characteristic may make them better suited to monitor minor changes in cartilage and joint health such as those expected from horses with chronic osteoarthritis.

Fibrinogen is an acute-phase protein produced by the liver in response to inflammation. It plays an important role in the inflammatory process by acting as the substrate for thrombin in the clotting cascade. Plasma fibrinogen concentrations are believed by some practitioners to be important in identifying inflammatory diseases (Andrews et al., 1994). Due to its presence in the blood, fibrinogen concentrations cannot indicate the specific location of the insult or injury. However, similar to other inflammatory compounds in the plasma like PGE_2, changes in the fibrinogen concentration can be used to indicate a change in the inflammatory status of an animal.

Beneficial Levels of Omega-3s

In order for n3s to have beneficial effects they must be present in the circulation and incorporated into tissues. Previously, researchers have supplemented the common n3 PUFA alpha-linolenic acid (ALA, C18:3) to evaluate the anti-inflammatory properties of long chain fatty acids. However, ALA must be converted into EPA

(C20:5) and then DHA (C22:6) by a desaturase enzyme before it can be useful to the animal. The conversion rate of ALA to EPA can vary between 0.2% and 21%, while the conversion of ALA to DHA varies between 0% and 9% (Burdge, 2004). Therefore it may be more effective to directly feed EPA and DHA, which are found in large amounts in fish and fish products, rather than products high in ALA such as flaxseed.

O'Connor et al. (2001) supplemented equine diets with fish oil containing 10.8% EPA (C20:5n3) and 8% DHA (C22:6n3), resulting in a 4.8 fold increase in plasma EPA and a 4.66 fold increase in plasma DHA after 63 days. Additionally, King et al. (2005) reported a significant increase in both plasma EPA and DHA after feeding an n3 supplement for 28 days. As determined through previous studies, a level of 3.2% plasma phospholipid EPA and DHA must be reached in order to be effective at suppressing inflammatory cytokines and eicosanoids (Cleland et al., 2003). This concentration has been found in numerous studies in which beneficial results were obtained. Based on research completed in this laboratory (Ross, 2006), horses consuming 15 g of EPA and 20 g of DHA daily will reach this required percentage.

Numerous studies have provided evidence that n3 PUFA supplementation may be effective at reducing inflammation associated with diseases such as arthritis. However, while some research has shown benefits involving subjective measures of joint disease, none has evaluated the concentrations of inflammatory markers in synovial fluid of arthritic joints. To fully support the theory that n3 PUFAs are beneficial in the arthritic joint, it is necessary to monitor changes within the joint during supplementation.

CHAPTER III

MATERIALS AND METHODS

Horses

Sixteen mature arthritic horses, of Quarter Horse and Thoroughbred breeding, were blocked by severity of arthritis, affected joints, and age, and randomly divided into two groups. Severity of arthritis was determined by radiographs, lameness as determined by weight distribution measured by force plates, and d 0 synovial fluid WBC concentration. In total the study observed twenty knees, five fetlocks, one stifle, and two hock joints from ten geldings and six mares with a mean age of seventeen (Table 1).

Table 1. Summary of joints observed in osteoarthritic horses with and without n3 PUFA supplementation.

Joint	Control	Treatment	Total observed	Number of horses observed
Knee	10	10	20	10
Fetlock	3	2	5	4
Stifle	1	0	1	1
Hock	0	2	2	1

Horses were housed in 10m x 20m dry lot pens at the Texas A&M University Horse Center. Routine farrier work, vaccinations and de-worming were consistent with farm protocols. Body weight was measured every 30 days to adjust feed intake as needed. All protocol for this study were within the guidelines set forth by the Institutional Agricultural Animal Care and Use Committee (AUP# 2006-85).

Diets

One group of horses (n = 8) was randomly designated to the control diet; the second group (n = 8) was assigned to the treatment diet. Horses received their respective dietary treatments for 90 days. Diets were fed at levels to maintain an optimal body condition score (BCS= 5-6). The control group received a 12% crude protein textured feed purchased from Producer's Cooperative Association, Bryan, Texas, at 1% BW per day. Treatment horses received the same mixed feed and two pelleted n3 supplements (JBS United Feeds Inc, Sheridan, Indiana). In order to maintain isocaloric treatment groups, the treatment horses consumed 0.86 kg less than 1% of BW in concentrate (Table 2). As seen in the Results section, analysis revealed the gross energy supplied by the treatment and control diets was statistically equal ($P < 0.05$). Supplement A was formulated to contain EPA and DHA at 2.8% of total fatty acids, and supplement B was formulated to contain 2.2% EPA and 3.8% DHA. Each treatment horse received 300 g of each supplement per day, supplying an additional 15 g EPA (C20:5n3) and 19.8 g DHA (C22:6n3) to the diet.

Horses were placed in individual stalls and fed the concentrate or concentrate/supplement ration in twice daily at 12-hour intervals. Concentrate and supplements were weighed out prior to each feeding, and supplements were mixed into the concentrate immediately before consumption. Intakes and refusals were measured daily, and all horses consumed 2.01 kg/hd/d of concentrate on average. Both diets included group-fed access to coastal hay at approximately 1.3% BW, and horses consumed approximately 6.12 kg/hd/d. Grain, hay, and supplements were sampled

randomly throughout the study and analyzed for n3 and n6 PUFA concentration (Tables on pages 22 and 23) and gross energy concentrations.

Table 2. Mean total daily intake in osteoarthritic horses with and without n3 PUFA supplementation (kg/hd/d).

Group	Concentrate[c]	Coastal Hay	Supplement A[d]	Supplement B[e]
Control	4.60[a]	6.12	--	--
Treatment	3.40[b]	6.12	0.3	0.3

[a,b] Values within columns lacking common superscripts differ by $P < 0.01$
[c] Textured feed containing 12% crude protein
[d] n3 supplement containing 2.8% EPA and DHA
[e] n3 supplement containing 2.2% EPA and 3.8% DHA

Sample Collection

Fasting blood samples were collected prior to the morning feeding on d 0 and at 14-day intervals for the remainder of the study via jugular veni-puncture. Plasma samples were collected into triplicate 5 ml evacuated tubes containing EDTA, and into a single 5 ml evacuated tube containing lithium heparin. On d 0, serum samples were collected into a single 10 ml evacuated tube without additives. Samples collected in heparin tubes were stored in a cooler at 4^{o}C for immediate fibrinogen analysis. All other samples collected were cooled on ice and centrifuged at 3200 rpm for 20 minutes. After separation, plasma and serum were pipetted into labeled 1.5 ml microcentrifuge tubes, where they were capped and stored upright at -20 ^{o}C. Prior to freezing, the PGE_2 inhibitor indomethacin was added at 10% of volume to the EDTA samples to be used for PGE_2 analysis.

Synovial fluid was collected from each horse, from at least one affected joint, on d 0, 30, 60, and 90. Joints were washed with a Betadine and water mix prior to arthrocentesis. All fluid was collected in 3 ml evacuated tubes containing EDTA and

immediately cooled on ice. Samples for cytokine analysis were centrifuged at 3200 rpm for 20 minutes, pipetted into microcentrifuge tubes and stored upright at -20 ^{o}C for later analysis. Samples used to determine synovial fluid WBC concentration were immediately cooled on ice and taken to the Texas Veterinary Medical Diagnostic Lab, College Station, TX, for analysis on a Celdyne 3700 cell counter.

Force plate analysis was conducted on d 0, 30, 60, and 90 to determine changes in weight distribution throughout the study according to procedures described by Hood et al. (2001). Four independent force plates (weight scales), one for each foot, were connected to a custom-designed computer system in a set of stocks. This system records the mean load, calculated as a percent of body weight, placed on each foot every 0.1 s over a 3 min period (Hood et al., 2001). Three readings were taken for each horse on each day**.** All horses were trailered to the force plates on the mornings of collection days prior to arthrocentesis to avoid discrepancies in lameness due to the procedure.

Laboratory Analysis

Dry matter content of concentrate and forage samples was determined. Feed samples were ground using a Wiley mill accommodated with a 1 mm mesh screen. Approximately 2.0 g of ground concentrate and forage sample were measured in triplicate into aluminum pans and placed in a drying oven (68°C) for 72 hours. Pans were removed and placed inside a dessicator for cooling and to prevent condensation. Sample analysis was repeated if standard error was greater than 5%. Dry sample weight was determined by subtracting sample weight after drying from the sample weight prior to drying. Dry matter percentage was calculated using the following formula:

$$\frac{\text{Dry sample weight (g)}}{\text{Wet sample weight (g)}} \text{ X } 100$$

Plasma fibrinogen concentration was determined within 24 h of blood collection. Fibrinogen concentration was determined using micro-hematocrit heat precipitation methods of Millar et al. (1971). Whole blood was harvested into collection tubes containing heparin. Two 75 mm heparinized micro-hematocrit capillary tubes (Fisher Scientific) were filled at least 50% with whole blood and sealed at one end using critoseal (Oxford Labware). The tubes were centrifuged for 5 minutes, then immediately transferred upright into a water bath (56 +/-1°C) for 3 min, and centrifuged for an additional 3 min. Using a digital caliper, the length of the fibrinogen column and total length of fibrinogen-plasma column was measured. Fibrinogen concentration (mg/dl) of each sample was determined using the following equation:

$$\frac{\text{Fibrinogen column}}{\text{Fibrinogen-plasma column}} \text{ X } 10{,}000$$

Plasma, serum, supplement, concentrate, and forage samples were analyzed for total fatty acid concentrations using a gas chromatograph (GC). All feed samples were dried prior fatty acid analysis, and 50-500 mg of dried samples were placed in screw cap tubes with Teflon lined caps. Two ml of benzene containing the internal standard, methyl tridecanoic acid (C13:0), was added to all tubes, along with 3 ml of methanolic-HCl. Nitrogen (N_2) gas was added, and samples were capped and vortexed. Tubes were then heated for 2 hr in an 80°C water bath. After heating, tubes were cooled to room temperature and 5 ml of 6% K_2CO_3 and 2 ml benzene was added; tubes were

vortexed. Tubes were then centrifuged at 500 rpm for 5 min. The upper organic solvent layer was then transferred to a GC vial. The autosampler obtained 1.0 μl of sample for analysis in the Supelco SP-2560 capillary column. Temperature at injection and detection was 260°C, and column flow rate was 1.1 ml/min. The Supelco 37 FAME mix (# 47885-U) was used as a standard.

Plasma was pipetted in 500 μl units in to 15 ml screw cap tubes for fatty acid analysis. Samples were freeze-dried overnight prior to analysis. One ml of benzene containing the internal standard (1000μg/ml methyl-C:13) was added and tubes were capped and vortexed. Four ml of Supelco B252, the BF_3-Methanol reagent, was added and tubes were gently mixed. Tubes were then tightly capped and incubated for 60 min at 60°C. After tubes cooled to room temperature, 4 ml of double distilled H_20 and 1 ml hexane was added. Tubes were vigorously mixed and centrifuged at 1000 rpm for 5 min. The upper solvent layer, approximately 1-2 ml, was transferred to a GC vial and 3-4 small crystals of anhydrous sodium sulfate were added to remove small amounts of water that may have remained in the sample. Fatty acid analysis of plasma was conducted using the same GC column and procedures used for feed analysis.

Gross energy content was determined on ground samples of concentrate, hay, and supplements. Samples were not dried before analysis. Approximately 1.0 g aliquots of concentrate and hay, and 0.5 g aliquots of supplement A and supplement B were measured. Gross energy was then determined on the aliquots by bomb calorimetery using a Par 6300 oxygen-bomb calorimeter (Parr Instrument CO., Moline, IL).

Plasma samples were analyzed for PGE_2 using enzyme-linked immunoassay (ELISA) kits (R&D systems, Minneapolis, MN). Plasma samples required 2, 5 and 10-fold dilutions depending on the severity of arthritis. The mean minimum detectable dose of PGE_2 was 27.5 pg/ml. Dilutions were made with a calibrator diluent provided by the kit prior to beginning the assay.

Synovial fluid IL-1 and TNF-alpha were determined using ELISA kits (Amersham Biosciences, Piscataway, NJ). Samples for IL-1 analysis required 2-fold dilutions, and samples analyzed for TNF-alpha required 5-fold dilutions. The mean minimum detectable dose was 0.1 pg/ml for both kits. Dilutions were made with a calibrator diluent provided by the kit prior to beginning the assay.

ELISA kits supplied all necessary reagents, and concentrations were read using a Biotek microplate reader (Biotek Instruments, Inc., Winooksi, VT)at 450nm. All assay samples, standards, and controls were run in duplicate unless otherwise stated and were repeated if the standard error was greater than 5%.

Statistical Analysis

All data was analyzed by analysis of variance (ANOVA) using STATA 8 statistical software (Stata Corp., College Station, TX). Due to the high variability between horses and large variation between groups at day 0, normalized values were compared by subtracting day 0 values from all measurements. Each parameter was analyzed for treatment, day, and treatment by day interactions.

CHAPTER IV

RESULTS

Horses

One horse from the control group was euthanized on d 60 due to problems unrelated to the study. The horse entered the study with laminitis, however, after d 30 the severity of the laminitis worsened. This increase in inflammation of the tissue in the foot increased plasma fibrinogen and PGE_2 beyond the range associated with normal variation. For this reason, all plasma data for this horse were removed. In addition, the laminitis affected the front limb opposite of that in which synovial fluid was obtained. Therefore, more weight was placed on the observed knee joint exposing the joint to conditions vastly different from those on d 0. Data from synovial fluid analysis were also removed.

Partial data from one horse in the treatment group were also removed from the study due to a worsening in laminitis. All plasma values were dropped; however, synovial fluid WBC concentration did not appear to be greatly affected, compared to d 0 values, so this data was kept.

Diet Analysis

The coastal hay contained more gross energy than expected (Table 3); however, each horse in the study was consuming similar amounts and therefore this difference was not a factor. Diets were formulated to be isocaloric, and the control horses were consuming 43.57 Mcal/hd/d compared to 41.77 Mcal/hd/day for the treatment group (Table 3). This difference is not significant ($P < 0.05$).

Table 3. Gross energy content of diet components (Mcal/kg) and daily mean gross energy intake (Mcal/hd/d) of osteoarthritic horses with and without n3 PUFA supplementation.

Component	Gross energy	Control[a]	Treatment[a]
Concentrate	4.05	18.63	13.77
Coastal Hay	4.08	24.94	24.94
Supplement A	5.07	---	1.52
Supplement B	5.13	---	1.54
Total		43.57	41.77

[a] Calculated by multiplying average daily intake and gross energy content of diet component.

Fatty acid concentrations in feeds are typically reported as a percent of total fatty acid content. However, it is also necessary to determine the actual amount of each fatty acid consumed by the horse to use in subsequent research or diet formulations. In order to do this, fatty acid profiles must be determined as a percent of the total diet. Tables 4 and 5 report the fatty acids in both forms.

Coastal hay was analyzed for fatty acid profile, and all results can be found in appendices 1 and 2**.** Hay was group-fed and all horses were offered similar amounts, therefore the fatty acids supplied by the hay do not differ between control and treatment groups.

As shown in Table 4, linoleic acid (C18:2n6) and alpha-linolenic acid (C18:3n3) were found in high concentrations in the concentrate, specifically 42.107% linoleic acid and 3.496% alpha-linolenic acid as a percent of total fatty acids. These results agree with previous research in our lab (Ross, 2006). Eicosapentaenoic acid (C20:5n3) was found in lower concentrations than expected in the supplements. It was found to be 0.096% and 0.066% of supplement A and B, respectively, while we were anticipating

levels closer to 2.5%. Concentrations of DHA (C22:6n3) are also slightly lower than expected, though not as low as EPA.

While the EPA concentration in the supplements was much lower than expected, the total n6:n3 ratios of both supplements are still lower than n3 sources used in other studies due to moderate DHA concentrations. Previous studies have fed oils or supplements at 6:1 or 5:1 n6:n3 ratio (Ross, 2006), while the supplements used in this research had ratios of 1.2:1 and 0.4:1 in A and B, respectively (Table 4).

Table 4. Fatty acid profile of concentrate and n3 supplements, reported as % of total fatty acids (% by weight).

Fatty acid	Concentrate	Supp. A	Supp. B
C18:2n6 (LA)	42.107	5.502	7.407
C18:3n6 (GLA)	0.001	0.002	0.004
C18:3n3 (ALA)	3.496	1.152	2.241
C20:3n6 (DGLA)	0.017	0.135	0.210
C20:4n6 (AA)	0.030	0.325	0.717
C20:5n3 (EPA)	0.113	0.096	0.066
C22:5n3 (DPA)	0.020	0.025	0.027
C22:6n3 (DHA)	0.165	3.732	17.931
n6:n3 ratio	11.0:1	1.2:1	0.4:1

Table 5. Fatty acid profile of concentrate and n3 supplements, reported as actual % of each diet component.

Fatty acid	Concentrate	Supp. A	Supp. B
C18:2n6 (LA)	2.430	0.575	1.075
C18:3n6 (GLA)	0.003	0.016	0.052
C18:3n3 (ALA)	0.202	0.121	0.325
C20:3n6 (DGLA)	0.001	0.014	0.031
C20:4n6 (AA)	0.002	0.034	0.104
C20:5n3 (EPA)	0.007	0.010	0.019
C22:5n3 (DPA)	0.001	0.003	0.004
C22:6n3 (DHA)	0.009	0.389	2.600

Intake of all fatty acids except EPA (C20:5n3) were different between groups ($P < 0.05$) (Table 6). This was due not only to the inclusion of supplements in the treatment diet, but also due to the increased concentrate in the control diet to maintain isocaloric diets. As expected, increases in grain intake in the control group resulted in a higher LA (C18:2n6) intake due to the higher concentration of this component. In addition, the amount of DHA (C22:6n3) consumed was much higher in the treatment group than the control. All other fatty acids were consumed in similar amounts when evaluated from a physiological perspective.

Table 6. Fatty acid consumption (g) of osteoarthritic horses with and without n3 PUFA supplementation, excluding those supplied by hay.

Group	LA 18:2 n6	GLA 18:3 n6	ALA 18:3 n3	DGLA 20:3n6	AA 20:4 n6	EPA 20:5 n3	DPA 22:5 n3	DHA 22:6 n3
Control	113.69 [a]	0.14 [a]	9.36[a]	0.05[a]	0.09 [a]	0.33	0.05[c]	0.42 [a]
Treatment	88.24 [b]	0.31 [b]	8.18 [b]	0.17 [b]	0.46 [b]	0.32	0.06 [d]	9.28 [b]

[a,b] Values within columns lacking common superscripts differ by $P < 0.01$
[c,d] Values within columns lacking common superscripts differ by $P < 0.05$

When n6:n3 ratios were compared, decreased LA (C18:2n6) and increased DHA (C22:6n3) intakes of horses in the treatment group vastly affected the n6:n3 ratios (Table 7).

Table 7. Total n6 and n3 fatty acids consumed (g), and n6:n3 ratios, of osteoarthritic horses with and without n3 PUFA supplementation, excluding those supplied by hay.

	n6s consumed	n3s consumed	n6:n3 ratio
Control	114.00[a]	10.20 [a]	11.0:1
Treatment	89.20 [b]	17.80 [b]	5.0: 1

[a,b] Values within columns lacking common superscripts differ $P < 0.01$

Plasma Fatty Acid Profiles

Mean plasma fatty acid concentrations are found in Table 8. Treatment horses had higher mean plasma concentrations of DHA (C22:6n3) and decreased concentrations of LA (C18:2n6) ($P < 0.05$). Linoleic acid concentrations were also lower on d 15, 60, and 90 in treatment horses ($P < 0.05$). Docosahexaenoic acid concentrations were significantly higher in treatment horses on all days except 0 and 75 ($P < 0.05$).

In addition, plasma AA (C20:4n6) was greater in the treatment horses, and EPA (C22:5n3) was decreased compared to control ($P < 0.05$). While mean ALA (C18:3n3) concentrations were not different between groups, treatment horses did have lower concentrations on d 45, 60, and 90.

The decreased LA (C18:2n6) concentration was mainly due to decreased concentrate intake of treatment horses. This reduction in n6 fatty acid intake plays a large role in decreasing the plasma n6:n3 ratio seen (Table 9). As noted above, the lowered n6:n3 ratio of the treatment diet was due in part to an increased DHA (C22:6n3) concentration. This was also reflected in the plasma values (Table 8).

Table 8. Mean plasma fatty acid concentration (μg/g) of osteoarthritic horses with and without n3 PUFA supplementation.

	d 0	d 15	d 30	d 45	d 60	d 75	d 90	Mean
C18:2n6 (LA)								
Control	821	796[a]	768	759	749[a]	724	768[a]	769[a]
Treatment	892	681[b]	647	635	591[b]	616	628[b]	670[b]
C18:3n6 (GLA)								
Control	5.6	4.4	3.7	2.9	3.7	3.6	5.5	4.2
Treatment	5.2	5.6	4.8	2.4	4.2	2.9	2.9	0.3
C18:3n3 (ALA)								
Control	36.3	19.5	17.6	16.6[a]	16.9[a]	12.4	17.9[a]	19.6
Treatment	36.6	13.8	13.1	13.1[b]	11.9[b]	11.5	12.8[b]	16.1
C20:3n6 (DGLA)								
Control	7.0	7.1[a]	4.5	6.1	5.9	6.4	8.4	6.5
Treatment	5.9	5.7[b]	5.4	6.6	6.3	6.6	7.0	6.2
C20:4n6 (AA)								
Control	1.7	1.4[a]	1.5[a]	2.4	1.7[a]	1.6	1.6	1.7[a]
Treatment	1.9	3.6[b]	3.2[b]	3.4	3.4[b]	3.0	4.2	3.2[b]
C20:5n3 (EPA)								
Control	2.7	3.0[a]	1.9	2.0	2.1	1.9	2.4	2.3[a]
Treatment	1.7	1.1[b]	1.5	1.3	1.9	1.0	1.0	1.4[b]
C22:5n3 (DPA)								
Control	2.9	2.5	2.5	3.1	2.5	2.8	2.6	2.7
Treatment	4.2	2.3	3.4	2.0	3.3	2.7	3.5	3.0
C22:6n3 (DHA)								
Control	5.2	4.6[a]	3.4[a]	4.1[a]	2.8[a]	2.4	2.0[a]	3.5[a]
Treatment	5.1	7.9[b]	10.3[b]	10.0[b]	7.9[b]	7.9	7.8[b]	8.4[b]

[a,b] Values within columns within the same fatty acid lacking common superscripts differ by $P < 0.05$

Total n3 concentration in the plasma was not different between groups (Table 9). A decreased n6:n3 ratio in the plasma was due to a decrease in mean plasma n6 concentration ($P < 0.05$).

Table 9. Plasma concentration (μg/g) of total n6 and n3 fatty acids of osteoarthritic horses with and without n3 PUFA supplementation.

n6			n3		
Day	Control	Treatment	Day	Control	Treatment
0	835	905	0	47.1	47.5
15	809[a]	696[b]	15	29.6	26.8
30	778	661	30	25.5	28.3
45	771	647	45	25.8	26.4
60	760[a]	605[b]	60	24.4	25.0
75	735	629	75	19.6	23.1
90	784[a]	642[b]	90	24.9	25.2
Mean	782[a]	683[b]	Mean	28.1	28.9
n6:n3 ratio	27:1	23:1			

[a,b] Values within rows lacking common superscripts differ by $P < 0.05$

Synovial Fluid WBC Concentration

A treatment effect on synovial fluid WBC concentration was observed when the data were normalized. Horses consuming n3 supplements experienced a significantly larger negative change in WBC concentration ($P < 0.05$) compared to control horses. Treatment horses experienced a change of -214 $\pm$ 55 cells/ml compared to 90 $\pm$ 144 cells/ml in the control group for the entire trial.

Horses consuming the control diet had increased WBC concentration on d 30; however, the concentration returns to near d 0 values by the next data collection at d 60 (Figure 1). The lack of a pre-trial adjustment period for all horses and abrupt changes in living conditions and diet could explain the increase in WBC concentration within the first 30 days. The concentration of synovial fluid WBC concentration is observed to decline consistently throughout the 90 d trial.

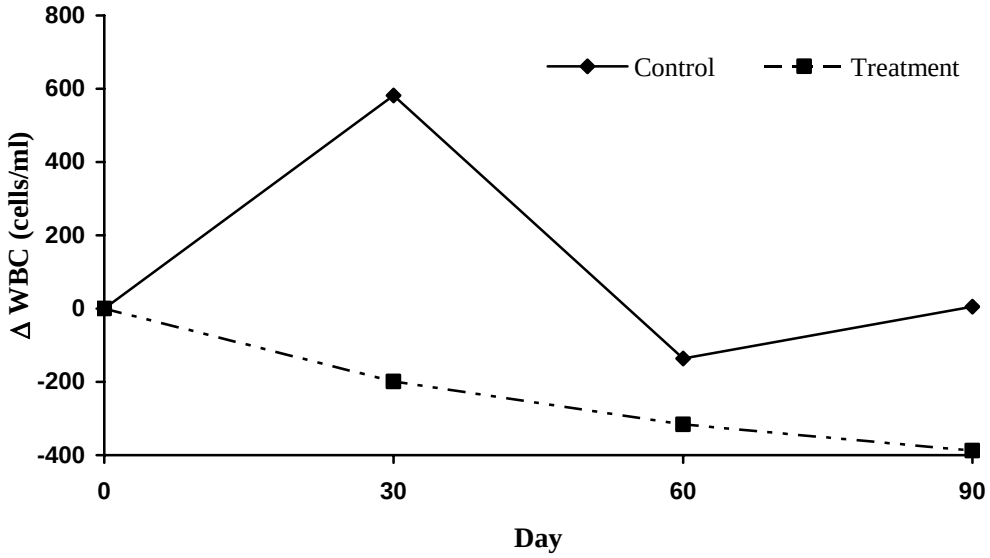

Figure 1. Mean change in synovial fluid WBC concentration in osteoarthritic horses with and without n3 PUFA supplementation.

Plasma PGE_2

There was a similar treatment effect on plasma PGE_2 concentrations with the treatment horses exhibiting a significantly larger negative change ($P < 0.05$) in plasma PGE_2 than the control horses. Mean plasma PGE_2 in the control horses increased by 540 $\pm$ 278 pg/ml, while the treatment horses experienced a mean change of -420 $\pm$ 247 pg/ml.

As illustrated in Figure 2, plasma PGE_2 concentrations of both groups separated from d 0 until d 30 at which point they parallelled each other from d 30 through d 90. The treatment group experienced a lower mean concentration throughout. The separation of means in the beginning of the study could have been due the time necessary for accumulation of fatty acids in to the tissue, as well as acclimation to the

study environment. After d 30, it appears concentrations level out, with both groups experiencing similar variations in mean concentration. The cause of increased mean concentrations between d 75 and 90 is unclear. Both groups experienced a similar increase, but while no significant differences on individual days are seen due to high variability, the treatment horses appear to experience a smaller increase in inflammation. The PGE_2 concentration in the plasma of control horses increased twice as much as the treatment group from d 75 to d 90 (Appendix 9).

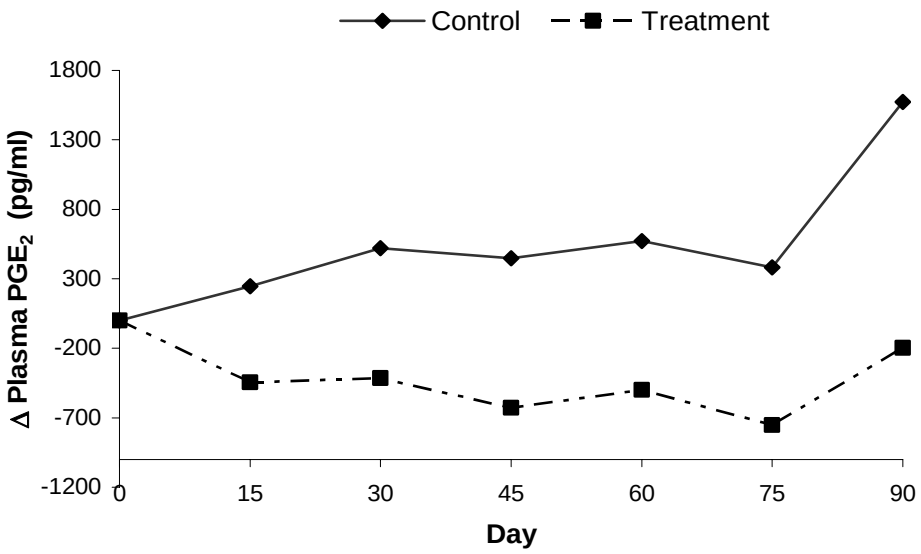

Figure 2. Mean change in plasma PGE_2 concentration in osteoarthritic horses with and without n3 PUFA supplementation.

Plasma Fibrinogen

Plasma fibrinogen was also measured at 15-day intervals throughout the study, beginning at d 0. A trend (P = 0.076) towards a larger negative change in plasma fibrinogen concentrations was observed in the treatment horses compared to the control

horses. Mean change in the control group for the entire trial was -9.5 ± 10.5 mg/dl compared to -40 + 13.3 mg/dl in treatment horses.

Due to an increase in fibrinogen concentration for both groups on d 15 (Figure 3), data were also analyzed from d 30 to d 90. In removing d 0 and d 15, analysis revealed concentrations between groups were significantly different ($P < 0.05$). After d 30, fibrinogen in both groups decreases with the control lowering to near d 0 values and the treatment horses experiencing a much larger negative change. Analogous to plasma $PGE_{2,}$ following the separation of mean concentrations the concentrations in both groups follow the same pattern.

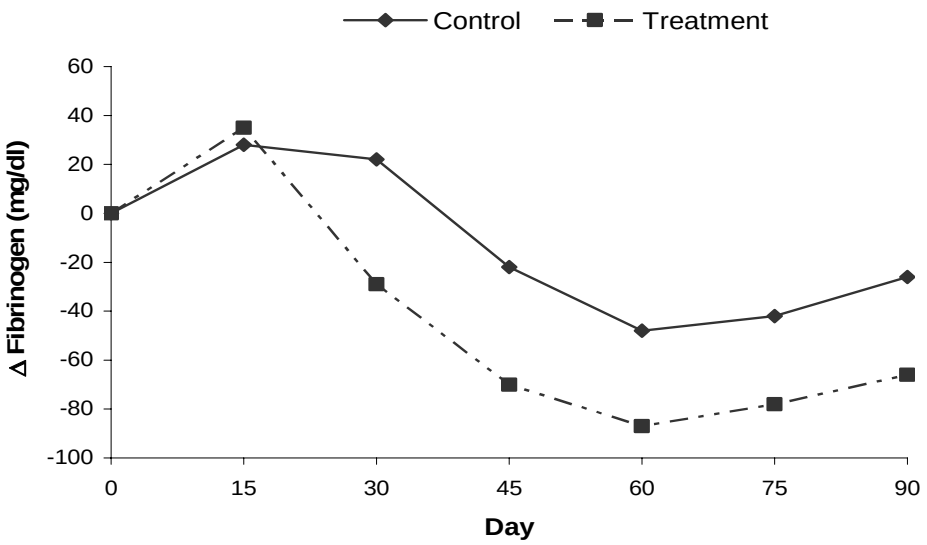

Figure 3. Mean change in plasma fibrinogen concentration in osteoarthritic horses with and without n3 PUFA supplementation.

Synovial Fluid Interleukin-1 and Tumor Necrosis Factor-α

Synovial fluid cytokine concentrations were not obtained due to difficulties with the assay kits or procedures. Numerous kits were used for both assays, as well as a variety of dilutions, including 1:10, 1:5, 1:2, and no dilution. Kits were stored at 4°C immediately upon arrival until use (within 4 wks). No data were ever obtained for TNF-α kits. Regardless of the dilution, the plate reader was unable to determine any changes in concentration. All readings were below the lowest point on the standard curve. The assays for IL-1 resulted in approximately half of the data points assayed for; however, these data points were later determined to be unusable. Similar to TNF-α, the concentrations were found to be very low regardless of the dilution used. Though little previous research has been done to verify normal IL-1 concentrations in equine synovial fluid, the values obtained were much lower than concentrations seen in normal or arthritic joints. Even those data points that could be duplicated appeared random and unrelated to other values obtained for the same horse on different days. Assay kit directions were followed closely, and kits were used within the recommended time frame. In addition, no synovial fluid was frozen and thawed more than once. Fluid was analyzed within 6 mo of collection. However, synovial fluid was not flash frozen in liquid nitrogen immediately upon collection, which is one difference compared other studies in which equine synovial fluid was assayed (Bertone et al., 2001). Instead, as mentioned previously, fluid was centrifuged, pipetted off, and frozen at -20 °C within one hour of collection. This variation may account for the lack of useable values obtained by the assay kits.

Force Plate Analysis

In measuring changes in weight distribution by force plate analysis no significant difference was seen between groups. Most horses on the project had arthritis in more than one joint, or unrelated lameness issues in the leg opposing the joint observed by arthrocentesis. Two horses were affected by laminitis in the opposite foot, and numerous others had moderate to severe arthritis in multiple joints in the same limb or the limb opposed to the joint being evaluated. Weight distribution in these horses was affected by the above issues and confounded force plate results. In analyzing for differences, data was sorted to remove all horses with confounding lameness issues. Data from seven horses remained, and ANOVA revealed a P-value of 0.12 when comparing weight distribution between groups (Table 10). These results could partially be explained by the small data set available for analysis, as well as a large variability between horses. While accounting for standard errors in evaluating overlap of the data, it is also important to evaluate the mean total force plate values that were observed. While significant only at P = 0.12, the mean force plate value observed for horses in the treatment group was 5 times larger than the control.

Table 10. Mean normalized weight (% of BW) placed on affected limb (± SE) of osteoarthritic horses with and without n3 supplementation.

Day	Control (n = 4)	Treatment (n = 3)
30	3.8 + 3.2	2.4 + 1.1
60	-0.9 +1.6	4.7 + 2.7
90	-2.1 + 0.08	2.85 + 1.8
Totals	0.5 + 1.1[a]	2.5 + 0.9[b]

[a,b] Values within rows lacking common subscripts differ (P = 0.12)

CHAPTER V

DISCUSSION

Fatty Acid Profiles

Treatment horses had significantly higher plasma DHA (C22:6n3) concentrations and lower LA (C18:2n6) concentrations. Arachidonic acid (C20:4n6) was also higher in treatment horses, and EPA (C20:5n3) was lower which did lead to an increase in the n6:n3 ratio in the plasma. However, as noted earlier, DHA and LA are the main fatty acids which affect the n6:n3 ratio of both the diets and plasma between groups. Therefore, the large difference in LA and DHA between groups greatly overwhelmed the negative effects of AA and EPA on the inflammatory state of treatment horses.

It is still unknown if one specific n3 fatty acid provides more anti-inflammatory benefits than others or if the ratio of total n6:n3 fatty acids is the key factor in reducing inflammation. Previous studies evaluating the effect of n3s on arthritis focused more on increasing concentrations of EPA and DHA (Woodward et al., 2005), and decreasing concentrations of AA to alter the inflammatory potential of cells. However, this study provided evidence that decreasing the total n6:n3 ratio of the diet by increasing only DHA while decreasing LA was just as effective at reducing production of inflammatory markers.

Inflammatory Markers

Horses fed supplemental n3s experienced a decrease in plasma PGE_2 and synovial fluid WBC concentration, as well as a trend towards decreased fibrinogen.

Fibrinogen is known to be a very broad marker of inflammation, and its presence in the blood indicates inflammation somewhere in the body. The exact origin is not able to be determined, and while normal fibrinogen concentrations are between 100-400 mg/dl (Andrews et al., 1994), normal concentration for each horse is quite variable. Small variations of fibrinogen due to reduced systemic inflammation would have been expected, but the differences seen here suggest the treatment horses may have experienced a decrease in total inflammation. The lower concentrations ($P< 0.05$) observed when data from d 0 and 15 were removed also provided evidence that treatment horses experienced reduced inflammation compared to control horses. Wilson (2003) found similar results in exercising horses. Horses fed a diet containing soybean oil as a source of n3s were observed to have lower fibrinogen concentrations compared to horses consuming a diet containing supplemental corn oil.

The negative change in plasma PGE_2 of the horses fed supplemental n3s also indicated a decline in inflammation somewhere in the body. Plasma PGE_2 levels also demonstrated the likelihood that a decline was caused by a change in the fatty acid profile of the animal. Because n6 fatty acids such as AA are known as the main precursor to inflammatory prostaglandins, a significant change in plasma PGE_2 concentrations was most likely due to a change in the fatty acid content of the cell membrane. The decreased PGE_2 concentration demonstrated a reduction in n6 fatty acids that may be explained by decreased concentrate intake and the addition of n3 supplements to the diet of treatment horses.

As previously noted, concentrations of plasma PGE_2 and fibrinogen in both groups varied similarly as the study progressed. After a separation of mean

concentrations, both plasma markers exhibited similar patterns throughout the trial, while treatment horses consistently had a lower mean concentration. This could be an indicator of a well-controlled study in which any outside variables affected both groups equally. Evidence of a controlled study increases the likelihood that the changes observed in inflammatory markers were caused by a treatment effect.

Synovial WBC concentration represents the health of the joint and joint fluid, and is not greatly affected by other inflammatory processes occurring in the body. Compounds found in the serum or plasma reflect systemic characteristics and changes, and many joint-specific markers from synovial fluid may be eliminated by the time they reach the vascular system (Lohmander et al.,1992). Although not the most sensitive marker of joint characteristics, synovial WBC concentration can be used to pinpoint changes specifically in joint health better than any compound found in the plasma. In this study, a significant reduction in synovial WBC concentration suggested that at least a part of the decrease in inflammation indicated by plasma markers did indeed occur in the joint. A lower WBC concentration in the synovial fluid of treatment horses provided evidence of a link between n3 fatty acids and the decreased joint inflammation and subsequent improvement of joint health. As mentioned previously, further evidence of decreased joint disease was not obtained from force plate analysis or synovial cytokine analyses, though the force plate data does suggest a tendency toward increased weight placement on arthritic limbs.

All markers of inflammation experienced some variation within the first 30 days of the study. Specifically, the control horses had increased mean WBC and PGE_2 concentrations at d 15 and 30, and both groups experienced increased fibrinogen

concentrations at d 15. Approximately 28 days of n3 supplementation is necessary to have any effect on the body (King et al., 2005), therefore changes before d 30 were most likely caused by other variables such as acclimation to diet and environment. Since arthritic horses were obtained for this study from various sources across a large geographical area, a standard adjustment period was not possible. Some horses from each group had more time to become accustomed to the study site than others. In future studies, a longer pre-trial period is recommended to minimize potential for any confounding variables. Also, many geriatric horses used in the study were not in good health or adequate body condition at the start of the trial. The improved feed quality and consumption for these horses improved their overall body condition regardless of which diet they were receiving.

Additional Applications

This study used mostly geriatric horses with osteoarthritis due to age; however, other classes of horses, such as performance horses, are also subject to joint disease. It has been shown that, while moderate exercise can stimulate bone and cartilage growth, intense exercise in animals leads to cartilage damage (Pap et al., 1998; Roos et al, 1995). Brama et al. (2000) found a significant increase in gross lesions in the cartilage as well as weakening of the collagen network in two-year old Thoroughbreds exercised in a typical race-training program. It is likely that performance horses experiencing increased workloads will acquire joint damage faster than horses on low to moderate exercise protocols. Therefore, the benefits of n3 PUFAs may also be applicable to the exercising horse. It is common in the performance equine industry to feed additional fat as an energy source. Research on polyunsaturated fatty acids has provided evidence

that n6s, such as that in corn oil, can have deleterious effects on system in the form of an increased ability to synthesize inflammatory mediators (Wilson, 2003). The supplements provided to the treatment group in this study supplied those horses with additional n3s in the form of DPA (C22:5) and allowed for a decreased concentrate intake, and therefore reduced n6 consumption. This resulted in a decrease in the plasma n6:n3 ratio which could be repeated to provide the same beneficial effects on joint health. At the same time, fat in the form of n3s can supply horses with the same positive effects as a traditional fat-supplemented diet such as decreased carbohydrate intake and reduced thermal load. Therefore, n3s can be useful, not only to the osteoarthritic horse, but also to horses currently experiencing intense exercise.

Osteoarthritis and other joint problems can certainly limit the usefulness of these animals after their competitive performance career has ended. Similar to geriatric horses, it is common for retired arthritic performance horses to be on daily medication such as phenylbutazone (bute) for pain management. As mentioned earlier, continual high doses of NSAIDs can cause other problems such as stomach ulcers (MacAllister et al., 1993). Because of this, an alternative treatment for inflammation caused by arthritis could be a major breakthrough for owners of retired performance horses.

In addition to decreased inflammation in the joint, the decrease in plasma inflammatory markers could also be accounted for by a decrease in inflammation in other parts of the body. Because plasma concentrations represent what is occurring systemically, it could be hypothesized that only part of the decrease in plasma fibrinogen and PGE_2 was due to a decline in arthritic inflammation. As mentioned earlier, most cell membranes in the body contain varying amounts of n6 and n3 PUFAs

depending on intake. Supplemental n3 consumption is expected to increase the amount of n3s in all cellular membranes. Therefore, additional n3s in the membranes of other cells in the body will decrease the inflammatory potential of those cells during insult or injury.

Omega-3 PUFAs have been tested for many anti-inflammatory uses, other than arthritis. Human researchers have investigated the possibility of using n3s for the treatment of cardiovascular disease, infertility, and to inhibit cancerous tumor growth. Mortality in men recovering from myocardial infarctions was decreased when patients consumed more fatty fish in their diet (Burr et al., 1989). Omega-3s have the potential to be of benefit to asthma patients as well, due to their negative effect on LTB_4 production when compared to n6s (Okamoto et al., 2000). Research has demonstrated the anti-inflammatory properties of n3s when consumed in adequate amounts in the diet. Further research is necessary to clarify the benefits of n3 supplementation on lameness due to arthritis as well as improving joint health and other physiological parameters in exercising horses.

CHAPTER VI

SUMMARY

Previous research has documented the positive effect of increased n3s in the diet on a variety of inflammatory responses. An increase in n3 consumption, as well as a decrease in n6 consumption has been shown to decrease the production of inflammatory eicosanoids and cytokines, along with causing a reduction in pain in both humans and other animals.

After 90 days of n3 supplementation, treatment horses experienced a decrease in inflammatory mediators both in the plasma and synovial fluid. A significantly larger negative change in synovial fluid WBC concentration and plasma PGE_2 concentration ($P < 0.05$) was seen in horses consuming supplemental n3s. The decreased concentration of inflammatory markers in the synovial fluid indicated a reduction of joint inflammation and subsequent increase in joint health. In addition, a trend ($P = 0.076$) toward a larger negative change in plasma fibrinogen was also observed in treatment horses. In analyzing weight distribution data from seven horses, a difference between groups was only significant at $P = 0.12$ suggesting the tendency of increased weight placement on arthritic limbs by treatment horses.

Treatment horses also experienced decreased plasma n6:n3 ratio through both the reduction of LA (C18:2n6) and addition of DHA (C22:6n3) in their diets. It is hypothesized that the decline in inflammation was due to a reduction of the total n6:n3 ratio in the plasma of treatment horses. These anti-inflammatory benefits can be valuable to the geriatric horse, along with exercising and retired performance horses.

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APPENDICES

APPENDIX 1. FATTY ACID PROFILE OF DIET COMPONENTS (% OF TOTAL FATTY ACIDS).

Fatty acid	Concentrate	Coastal Hay	Supp. A	Supp. B
C18:2n6 (LA)	42.11	18.20	5.50	7.41
C18:3n6 (GLA)	0.001	0.005	0.002	0.004
C18:3n3 (ALA)	3.50	15.62	1.16	2.24
C20:3n6 (DGLA)	0.02	0.08	0.14	0.21
C20:4n6 (AA)	0.03	0.20	0.32	0.72
C20:5n3 (EPA)	0.11	0.72	0.10	0.07
C22:5n3 (DPA)	0.02	0.08	0.03	0.03
C22:6n3 (DHA)	0.17	1.41	3.73	17.93
n6:n3 ratio	11.0:1	1.0:1	1.2:1	0.4:1

APPENDIX 2. FATTY ACID PROFILE OF DIET COMPONENTS (% OF DIET COMPONENT).

Fatty acid	Concentrate	Coastal Hay	Supp. A	Supp. B
C18:2n6 (LA)	2.430	0.231	0.575	1.075
C18:3n6 (GLA)	0.003	0.006	0.016	0.052
C18:3n3 (ALA)	0.202	0.190	0.121	0.325
C20:3n6 (DGLA)	0.001	0.001	0.014	0.031
C20:4n6 (AA)	0.002	0.003	0.034	0.104
C20:5n3 (EPA)	0.007	0.009	0.010	0.019
C22:5n3 (DPA)	0.001	0.002	0.003	0.004
C22:6n3 (DHA)	0.009	0.017	0.389	2.600

APPENDIX 3. TOTAL FATTY ACID PROFILE OF DIET COMPONENTS (% OF DIET COMPONENT).

Component	C6:0	C8:0	C10:0	C11:0	C12:0	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0
Grain	0.000	0.004	0.000	0.001	0.001	0.02	0.00	0.00	0.001	0.95	0.02	0.01
Hay	0.000	0.002	0.000	0.001	0.017	0.05	0.00	0.01	0.001	0.34	0.05	0.01
Supp A	0.000	0.003	0.000	0.007	0.026	1.48	0.01	0.13	0.002	3.21	1.50	0.14
Supp B	0.000	0.002	0.000	0.006	0.080	1.35	0.01	0.10	0.003	2.86	1.46	0.11

Component	C17:1	C18:0	C18:1n9t	C18:1n11	C18:1n9c	C18:1n7	C18:2n6t	C18:2n6c	C18:3n6	C18:3n3	C20:0
Grain	0.00	0.15	0.05	0.000	1.69	0.06	0.004	2.39	0.003	0.206	0.001
Hay	0.00	0.05	0.02	0.000	0.14	0.02	0.006	0.22	0.006	0.193	0.001
Supp. A	0.03	0.55	0.07	0.038	1.12	0.39	0.076	0.49	0.016	0.121	0.020
Supp. B	0.03	0.44	0.01	0.120	1.65	0.39	0.024	1.04	0.052	0.325	0.033

Component	C20:1	C20:2	C20:3n6	C20:4n6	C20:5n3	C21:0	C22:0	C22:5n3	C22:6n3	C24:0	C24:1
Grain	0.046	0.002	0.001	0.001	0.006	0.002	0.001	0.001	0.009	0.008	0.024
Hay	0.015	0.008	0.001	0.003	0.012	0.002	0.003	0.001	0.018	0.016	0.017
Supp. A	0.119	0.091	0.014	0.033	0.009	0.012	0.005	0.003	0.381	0.267	0.010
Supp. B	0.136	0.364	0.030	0.104	0.008	0.006	0.004	0.002	2.582	1.074	0.018

APPENDIX 4. INDIVIDUAL FATTY ACID INTAKE (g) OF EVERY HORSE, EXCLUDING HAY.

Horse	Treatment	C8	C11:0	C12:0	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0
1A	Y	0.13	0.07	0.35	9.06	0.06	0.69	0.04	45.29	9.75	2.75
2A	Y	0.13	0.07	0.35	9.08	0.06	0.69	0.04	46.24	9.77	2.82
3A	Y	0.18	0.08	0.36	9.32	0.06	0.69	0.06	57.83	10.01	3.67
4A	Y	0.19	0.08	0.36	9.38	0.06	0.69	0.06	60.58	10.07	3.87
5A	Y	0.13	0.07	0.35	9.06	0.06	0.69	0.04	45.29	9.75	2.75
6A	Y	0.18	0.08	0.36	9.31	0.06	0.69	0.06	57.16	10.00	3.62
7A	Y	0.15	0.07	0.35	9.15	0.06	0.69	0.05	49.75	9.84	3.07
8A	Y	0.12	0.07	0.35	9.04	0.06	0.69	0.04	44.15	9.73	2.66
1B	N	0.20	0.05	0.05	1.00	0.00	0.00	0.05	47.60	1.00	3.51
2B	N	0.16	0.04	0.04	0.82	0.00	0.00	0.04	38.86	0.82	2.86
3B	N	0.21	0.05	0.05	1.06	0.00	0.00	0.05	50.26	1.06	3.70
4B	N	0.20	0.05	0.05	1.02	0.00	0.00	0.05	48.36	1.02	3.56
5B	N	0.18	0.04	0.04	0.89	0.00	0.00	0.04	42.18	0.89	3.11
6B	N	0.20	0.05	0.05	0.99	0.00	0.00	0.05	47.03	0.99	3.47
7B	N	0.16	0.04	0.04	0.80	0.00	0.00	0.04	38.19	0.80	2.81
8B	N	0.18	0.05	0.05	0.91	0.00	0.00	0.05	43.13	0.91	3.18

APPENDIX 4 CONTINUED.

Horse	Treatment	C17:1	C18:0	C18:1n9	C18:1n11	C18:1n7	18:2n6	18:3n3	18:3n6	C20:0	C20:1
1A	Y	0.18	10.28	58.40	0.47	4.05	74.18	7.02	0.29	0.18	2.05
2A	Y	0.18	10.43	60.15	0.47	4.11	76.61	7.22	0.29	0.19	2.09
3A	Y	0.18	12.26	81.50	0.47	4.84	106.25	9.66	0.33	0.20	2.64
4A	Y	0.18	12.69	86.57	0.47	5.02	113.30	10.24	0.34	0.20	2.77
5A	Y	0.18	10.28	58.40	0.47	4.05	74.18	7.02	0.29	0.18	2.05
6A	Y	0.18	12.15	80.27	0.47	4.80	104.55	9.52	0.33	0.20	2.61
7A	Y	0.18	10.98	66.62	0.47	4.33	85.60	7.96	0.30	0.19	2.26
8A	Y	0.18	10.10	56.30	0.47	3.98	71.26	6.78	0.29	0.18	1.99
1B	N	0.00	7.52	87.68	0.00	3.01	121.74	10.02	0.15	0.05	2.25
2B	N	0.00	6.14	71.58	0.00	2.45	99.39	8.18	0.12	0.04	1.84
3B	N	0.00	7.94	92.58	0.00	3.17	128.55	10.58	0.16	0.05	2.38
4B	N	0.00	7.64	89.08	0.00	3.05	123.69	10.18	0.15	0.05	2.29
5B	N	0.00	6.66	77.70	0.00	2.66	107.89	8.88	0.13	0.04	2.00
6B	N	0.00	7.43	86.63	0.00	2.97	120.29	9.90	0.15	0.05	2.23
7B	N	0.00	6.03	70.35	0.00	2.41	97.69	8.04	0.12	0.04	1.81
8B	N	0.00	6.81	79.45	0.00	2.72	110.32	9.08	0.14	0.05	2.04

APPENDIX 4 CONTINUED.

Horse	Treatment	C20:2	20:3n6	20:4n6	20:5n3	C21:0	C22:0	22:5n3	22:6n3	C24:0	C24:1
1A	Y	1.46	0.16	0.45	0.27	0.11	5.55	0.05	9.22	4.28	0.85
2A	Y	1.46	0.16	0.45	0.28	0.11	5.65	0.05	9.23	4.29	0.88
3A	Y	1.50	0.17	0.47	0.37	0.14	6.87	0.06	9.34	4.40	1.21
4A	Y	1.50	0.18	0.48	0.39	0.14	7.16	0.07	9.37	4.42	1.29
5A	Y	1.46	0.16	0.45	0.27	0.11	5.55	0.05	9.22	4.28	0.85
6A	Y	1.49	0.17	0.47	0.36	0.14	6.80	0.06	9.34	4.39	1.19
7A	Y	1.47	0.17	0.46	0.31	0.12	6.02	0.05	9.27	4.32	0.98
8A	Y	1.45	0.16	0.44	0.27	0.11	5.43	0.05	9.21	4.27	0.82
1B	N	0.15	0.05	0.10	0.35	0.10	5.01	0.05	0.45	0.45	1.35
2B	N	0.12	0.04	0.08	0.29	0.08	4.09	0.04	0.37	0.37	1.10
3B	N	0.16	0.05	0.11	0.37	0.11	5.29	0.05	0.48	0.48	1.43
4B	N	0.15	0.05	0.10	0.36	0.10	5.09	0.05	0.46	0.46	1.37
5B	N	0.13	0.04	0.09	0.31	0.09	4.44	0.04	0.40	0.40	1.20
6B	N	0.15	0.05	0.10	0.35	0.10	4.95	0.05	0.45	0.45	1.34
7B	N	0.12	0.04	0.08	0.28	0.08	4.02	0.04	0.36	0.36	1.09
8B	N	0.14	0.05	0.09	0.32	0.09	4.54	0.05	0.41	0.41	1.23

APPENDIX 5. AVERAGE INTAKE OF INDIVIDUAL FATTY ACIDS (g) BY GROUP, EXCLUDING HAY.

Group	C8	C11:0	C12:0	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0
Treatment	0.15	0.07	0.35	9.18	0.06	0.69	0.05	50.78	9.87	3.15
Control	0.19	0.05	0.05	0.94	0.00	0.00	0.05	44.45	0.94	3.28

Group	C17:1	C18:0	C18:1n9	C18:1n11	C18:1n7	18:2n6	18:3n3	18:3n6	C20:0	C20:1
Treatment	0.18	11.14	68.52	0.47	4.40	88.24	8.18	0.31	0.19	2.31
Control	0.00	7.02	81.88	0.00	2.81	113.69	9.36	0.14	0.05	2.11

Group	C20:2	20:3n6	20:4n6	20:5n3	C21:0	C22:0	22:5n3	22:6n3	C24:0	C24:1
Treatment	1.47	0.17	0.46	0.32	0.12	6.13	0.06	9.28	4.33	1.01
Control	0.14	0.05	0.09	0.33	0.09	4.68	0.05	0.42	0.42	1.26

APPENDIX 6. NORMALIZED PLASMA CONCENTRATIONS OF n6 AND n3 FATTY ACIDS.

Fatty acid	Control	Treatment
C18:2 n6(LA)		
d 15	-24.9	-1.3
d 30	-52.5	-1.9
d 45	-61.3	-2.7
d 60	-71.4	-2.0
d 75	-97.1	-2.1
d 90	-52.6	-0.1
Mean	-51.4[a]	-1.4
C18:3 n6 (GLA)		
d 15	-1.3	0.5
d 30	-1.9	-0.4
d 45	-2.7	-2.7
d 60	-2.0	-0.9
d 75	-2.1	-2.3
d 90	-0.1	-2.2
Mean	-1.4	-1.2
C18:3 n3 (ALA)		
d 15	-16.8	-22.8
d 30	-18.6	-23.5
d 45	-19.6	-23.5
d 60	-19.3	-24.7
d 75	-23.8	-25.1
d 90	-18.4	-23.8
Mean	-16.6	-20.5
C20:3 (n6)		
d 15	0.1	-0.2
d 30	-2.6	-0.5
d 45	-1.0	0.7
d 60	-1.1	0.4
d 75	-0.7	0.7
d 90	-1.3	1.1
Mean	-0.6	0.3

Fatty acid	Control	Treatment
C:20:4 n6 (AA)		
d 15	- 0.4 [a]	1.7
d 30	-0.2	1.3
d 45	0.7	1.5
d 60	-0.4	1.5
d 75	0.0	1.1
d 90	-0.1	2.3
Mean	-0.1	1.3[b]
C20:5 n3 (EPA)		
d 15	0.1	-0.6
d 30	-0.3	-0.1
d 45	-2.2[a]	-0.4
d 60	-0.5	0.2
d 75	-0.4	-0.7
d 90	-2.0	-0.6
Mean	-0.7	-0.3
C22:6 n3 (DHA)		
d 15	-0.7[a]	4.6
d 30	-1.8[a]	5.3
d 45	-1.1[a]	5.0
d 60	-2.4[a]	2.9
d 75	-2.8[a]	2.9
d 90	-3.2[a]	2.8
Mean	-1.7[a]	3.3[b]
C22:5 n3 (DPA)		
d 15	-0.4	-1.9
d 30	-0.4	-0.8
d 45	0.2	-2.2
d 60	-0.4	-0.9
d 75	-0.1	-1.5
d 90	-0.3	-0.7
Mean	-0.2[a]	-1.1[b]

[a,b] Values between groups with differing superscripts differ $P < 0.05$.

APPENDIX 7. PLASMA FATTY ACID PROFILE OF EACH HORSE, REPORTED AS g/g OF PLASMA.

Horse	Trt	Day	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1
1A	Y	0	42.5	11.0	16.3	2.9	835.1	71.1	19.3	10.8
1A	Y	15	18.5	8.8	16.0	4.2	240.8	28.9	12.0	8.8
1A	Y	30	18.5	8.8	16.0	4.2	240.8	28.9	12.0	8.8
1A	Y	45	17.4	8.5	15.1	4.5	234.7	28.4	13.5	6.7
1A	Y	60	17.2	15.2	12.8	4.4	193.4	22.6	10.1	5.9
1A	Y	75	19.9	4.0	13.4	3.6	225.7	26.9	11.4	5.0
1A	Y	90	15.1	6.9	14.7	3.1	219.1	28.2	10.3	5.4
2A	Y	0	46.4	12.9	10.9	4.3	924.5	81.6	22.6	12.5
2A	Y	15	10.0	4.0	13.1	2.9	183.1	18.3	9.9	4.7
2A	Y	30	14.8	6.6	14.2	4.4	161.2	19.0	10.7	6.5
2A	Y	45	11.0	11.0	12.2	4.9	161.9	21.1	13.8	6.8
2A	Y	60	16.3	7.8	10.0	5.7	147.6	21.2	11.8	4.9
2A	Y	75	11.9	4.4	9.8	1.9	145.2	17.0	11.4	0.7
2A	Y	90	12.0	43.8	47.1	1.7	138.3	15.0	8.2	3.0
3A	Y	0	14.2	5.0	9.8	3.6	241.1	26.5	11.7	6.9
3A	Y	15	15.1	7.9	16.1	5.9	204.9	19.8	15.6	6.9
3A	Y	30	14.5	4.7	12.9	3.6	219.0	19.3	10.2	6.2
3A	Y	45	13.3	6.3	11.3	3.6	202.7	17.4	8.6	7.5
3A	Y	60	10.9	4.2	12.2	2.9	186.7	15.7	9.3	7.1
3A	Y	75	11.9	8.1	8.3	3.0	192.4	18.2	13.2	8.0
3A	Y	90	11.4	3.9	10.0	0.9	191.8	18.1	16.6	4.2
4A	Y	0	18.9	7.4	9.9	4.4	237.1	27.6	9.8	8.8
4A	Y	15	15.0	5.3	7.2	2.0	193.3	16.1	9.1	3.7
4A	Y	30	14.3	4.0	6.4	2.1	168.6	14.2	11.1	5.9
4A	Y	45	13.7	6.1	9.2	0.4	186.1	13.7	13.8	4.6
4A	Y	60	11.3	7.3	6.7	0.0	182.2	19.0	15.7	5.9
4A	Y	75	13.6	7.0	5.5	2.7	170.1	17.1	10.2	5.2
4A	Y	90	15.9	7.0	6.4	0.4	187.0	20.8	11.2	3.7

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1
5A	Y	0	15.1	6.3	6.2	4.6	167.3	23.7	9.1	6.3
5A	Y	15	15.4	5.2	10.7	1.9	147.2	21.7	13.4	7.3
5A	Y	30	15.0	16.7	13.9	5.3	136.4	19.3	10.1	7.5
5A	Y	45	11.7	6.0	7.7	2.5	127.1	18.4	11.3	6.6
5A	Y	60	9.5	6.2	7.7	1.9	110.7	15.2	2.2	5.5
5A	Y	75	11.8	7.7	6.7	2.2	112.3	15.4	9.5	1.4
5A	Y	90	14.4	3.4	11.9	3.2	132.9	20.1	9.6	5.0
6A	Y	0	11.4	2.6	12.1	4.2	182.7	21.8	9.9	5.3
6A	Y	15	17.2	6.9	13.9	2.0	176.8	16.5	9.0	6.4
6A	Y	30	16.8	7.0	11.6	3.2	192.3	18.6	8.4	5.8
6A	Y	45	15.3	5.0	14.7	1.2	178.0	18.6	13.3	4.1
6A	Y	60	13.7	5.9	9.5	4.2	166.5	16.2	14.2	3.7
6A	Y	75	13.0	4.4	7.2	3.1	160.8	14.0	8.5	5.1
6A	Y	90	14.8	6.1	3.8	4.0	173.5	17.6	9.5	3.9
7A	Y	0	11.0	4.7	11.0	4.8	203.2	24.7	36.9	10.3
7A	Y	15	14.5	7.9	16.6	2.8	209.5	26.6	17.0	6.3
7A	Y	30	15.1	7.2	12.4	4.5	171.9	20.9	13.4	5.4
7A	Y	45	12.2	4.9	11.1	3.0	158.0	20.4	12.7	6.1
7A	Y	60	14.8	6.7	13.4	1.9	152.5	19.2	12.9	5.4
7A	Y	75	10.1	10.0	8.6	2.9	139.4	16.2	9.4	4.0
7A	Y	90	10.4	6.6	8.2	2.9	162.0	18.2	12.1	5.9
8A	Y	0	7.8	4.4	11.1	5.1	190.0	12.8	12.5	6.3
8A	Y	15	14.2	7.5	12.6	1.9	167.4	18.2	10.8	5.4
8A	Y	30	15.9	7.9	11.6	1.1	195.7	26.4	13.9	5.7
8A	Y	45	16.2	6.8	17.0	2.8	197.6	24.1	14.9	4.9
8A	Y	60	18.6	5.6	10.7	3.5	196.1	23.8	13.2	6.5
8A	Y	75	15.0	4.6	17.5	3.2	206.8	20.6	10.5	5.8

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1
8A	Y	90	12.5	7.7	3.3	5.5	179.4	21.5	10.9	4.7
1B	N	0	10.8	3.8	10.6	3.2	153.8	19.9	9.5	7.9
1B	N	15	12.7	5.6	12.9	1.6	180.6	15.2	12.3	6.5
1B	N	30	9.5	4.0	7.3	2.6	158.9	11.8	10.1	4.4
1B	N	45	12.4	5.6	11.3	4.6	183.5	12.0	11.8	8.5
1B	N	60	13.1	5.7	7.4	2.6	196.3	15.8	16.5	12.5
1B	N	75	7.0	5.3	3.0	2.8	151.7	12.0	9.2	7.6
1B	N	90	12.7	66.5	16.8	4.9	166.6	11.6	11.0	5.7
2B	N	0	13.0	6.6	10.1	2.2	180.9	18.9	13.2	6.7
2B	N	15	19.2	7.5	11.1	5.1	228.7	13.5	16.8	6.2
2B	N	30	12.2	8.6	8.4	2.3	186.5	13.3	11.9	8.6
2B	N	45	11.3	4.8	6.6	3.2	188.8	13.8	13.9	8.5
2B	N	60	12.3	7.2	10.5	2.0	212.1	15.3	11.9	10.5
2B	N	75	10.5	6.6	9.1	2.2	180.2	11.8	12.2	5.5
2B	N	90	16.8	8.5	7.4	2.9	198.3	15.8	12.6	5.1
3B	N	0	13.8	1.4	13.2	5.8	176.8	20.6	11.2	8.8
3B	N	15	16.5	5.3	13.3	2.8	191.0	15.8	11.3	5.6
3B	N	30	10.0	1.8	8.5	4.7	150.5	7.4	8.6	4.1
3B	N	45	18.9	6.6	12.9	3.7	176.8	9.8	10.6	3.3
3B	N	60	11.0	8.3	10.5	3.7	165.7	11.6	9.1	7.2
3B	N	75	9.3	5.3	7.2	1.5	147.6	11.2	10.9	7.4
3B	N	90	11.8	5.5	9.3	3.3	178.3	14.3	11.3	9.2
5B	N	0	19.6	6.4	11.6	3.7	219.2	17.0	14.5	7.8
5B	N	15	14.8	6.2	8.4	3.2	201.0	12.3	10.8	5.5
5B	N	30	11.0	5.8	12.4	4.0	203.0	14.5	12.0	7.6
5B	N	45	12.9	6.6	9.9	3.6	189.4	12.0	8.9	5.1

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1
5B	N	60	11.3	6.4	10.4	40.3	222.3	16.0	8.4	6.9
5B	N	75	11.7	4.8	8.0	3.9	193.8	14.9	9.8	6.7
5B	N	90	9.5	9.0	5.7	0.7	206.6	11.9	10.4	5.1
6B	N	0	11.4	4.6	10.6	2.6	154.6	12.7	12.7	5.8
6B	N	15	10.2	6.8	10.0	1.2	173.4	11.6	13.2	6.6
6B	N	30	16.5	4.3	7.7	0.0	153.2	8.5	14.0	5.3
6B	N	45	14.1	5.5	7.7	4.5	156.4	9.5	14.4	2.9
6B	N	60	11.2	10.1	9.3	2.6	175.4	9.2	10.3	6.6
6B	N	75	8.7	5.9	5.4	2.5	150.5	8.6	12.7	2.8
6B	N	90	7.7	7.8	6.4	2.7	157.8	7.7	14.4	4.3
7B	N	0	51.9	15.7	8.7	2.7	971.5	72.1	26.8	14.0
7B	N	15	14.5	7.4	11.2	2.1	247.2	19.3	9.6	6.8
7B	N	30	18.5	6.1	11.9	4.0	270.6	24.4	14.2	6.2
7B	N	45	17.7	6.6	8.6	1.3	244.4	15.8	10.5	6.4
7B	N	60	10.2	5.4	9.7	2.3	203.9	14.9	9.3	5.2
7B	N	75	12.2	5.5	9.4	1.7	219.1	12.0	8.2	4.4
7B	N	90	16.1	6.0	1.7	0.5	226.7	15.2	9.1	0.2
8B	N	0	17.9	5.9	10.1	2.3	192.5	15.9	13.3	6.0
8B	N	15	9.2	7.4	7.1	4.4	183.0	7.8	8.4	4.9
8B	N	30	17.3	5.9	7.9	4.7	257.0	13.7	14.4	6.9
8B	N	45	11.7	6.2	9.1	2.6	206.1	12.1	13.6	3.8
8B	N	60	11.0	5.6	9.7	2.8	204.6	10.2	15.6	4.5
8B	N	75	13.9	5.1	8.1	2.9	219.3	10.7	12.4	6.4
8B	N	90	11.5	4.2	20.4	20.6	185.9	14.1	11.5	4.9

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C18:0	C18:1n9t	C18:1n11	C18:1n9c	C18:1n7	C18:2n6t	C18:2n6c	C18:2n6
1A	Y	0	370.7	6.2	0	410.5	34.8	3.3	1358.2	1361.5
1A	Y	15	237.6	9.9	0	118.6	21.9	5.2	861.5	866.7
1A	Y	30	237.6	9.9	0	118.6	21.9	5.2	861.5	866.7
1A	Y	45	230.4	13.6	0	113.4	23.2	2.7	806.2	808.9
1A	Y	60	194.7	47.6	0	173.4	49.2	6	687.8	693.8
1A	Y	75	199.5	8.6	0	113.4	23.3	2.6	790	792.6
1A	Y	90	201.2	8.5	0	105.5	19.2	4.4	703.3	707.7
2A	Y	0	375.4	4.7	0	528	41.8	5.8	1385.1	1390.9
2A	Y	15	170.2	6	0	95.5	17.8	7.9	611.1	619
2A	Y	30	163	6.2	0	79.2	17.1	3.6	537.6	541.2
2A	Y	45	162.9	10.4	0	79.4	17.9	3.1	517.4	520.5
2A	Y	60	153.5	10.6	0	73	17.3	5.2	470.7	475.9
2A	Y	75	136.6	6.2	0	71	14.4	1.3	454.1	455.4
2A	Y	90	139.8	1.6	0	69.3	15.9	2.5	464.6	467.1
3A	Y	0	216.5	3.4	3.6	126.5	14.8	5.6	663	668.6
3A	Y	15	190.7	6.5	0	81.6	19.2	5	595.8	600.8
3A	Y	30	164.8	6.9	0	76.1	15	1.9	506.5	508.4
3A	Y	45	154.2	2.1	0	66.8	13.4	4.1	504.3	508.4
3A	Y	60	139.2	3.1	0	62.8	11.5	3.9	457.9	461.8
3A	Y	75	145.1	6.2	0	65.8	15.4	6.9	481.6	488.5
3A	Y	90	162.8	2	0	74.4	15.4	6.3	553.6	559.9
4A	Y	0	223.4	3.4	0	222	15.2	4	663.6	667.6
4A	Y	15	192.2	5	0	103.7	14.9	4	646.6	650.6
4A	Y	30	166.3	3.5	0	89	12.9	5.8	561.8	567.6
4A	Y	45	174.9	3.1	0	96.1	13.7	2.2	598.4	600.6
4A	Y	60	177.1	2.8	0	91.8	14	1.2	626.2	627.4
4A	Y	75	173.3	2.7	0	92.5	14.2	3.4	610.9	614.3
4A	Y	90	185.2	2.2	0	109.2	15.8	5.5	632	637.5

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C18:0	C18:1n9t	C18:1n11	C18:1n9c	C18:1n7	C18:2n6t	C18:2n6c	C18:2n6
5A	Y	0	189.7	9.1	0	106.4	17.7	4.2	719.2	723.4
5A	Y	15	210	11.4	0	115.1	18	2.4	747.6	750
5A	Y	30	178.5	8.3	0	96.8	17.5	6.4	717.1	723.5
5A	Y	45	177.3	3.7	0	90	15.2	4	676.2	680.2
5A	Y	60	183.2	14.3	0	95.4	16.4	8.1	709.4	717.5
5A	Y	75	175.4	1.5	0	95	16.8	8.5	700.9	709.4
5A	Y	90	236	9.7	3.4	148.3	11.9	3.9	670.9	674.8
6A	Y	0	175.7	10	0	92.2	13.9	4.5	612.3	616.8
6A	Y	15	174.9	9.7	0	105.6	14	4.8	633.1	637.9
6A	Y	30	167.9	7.5	0	95.3	13.9	7.4	616.8	624.2
6A	Y	45	152.8	8.3	0	86	11.7	3.9	546.4	550.3
6A	Y	60	157.9	2.3	0	79.1	10.5	5.6	552.4	558
6A	Y	75	162	3.3	0	93.4	11.9	4.9	605.1	610
6A	Y	90	208.2	3.6	0	107.6	15.8	8.8	758.8	767.6
7A	Y	0	188.5	11.2	0	104.8	21.8	3.3	683.8	687.1
7A	Y	15	168.1	6.3	0	80.7	18.6	4.5	599.3	603.8
7A	Y	30	146.8	10.9	0	71.4	17.1	3.1	550.6	553.7
7A	Y	45	130.6	5.8	0	70.2	14.4	5.3	483.5	488.8
7A	Y	60	134.8	1.8	0	66.9	12.1	5.7	509.2	514.9
7A	Y	75	159.2	13.3	0	74.9	17.2	7.4	608.1	615.5
7A	Y	90	188.5	4.5	3.6	106.7	10.6	5.9	796.9	802.8
8A	Y	0	167.1	5.6	0	77	14.1	2.9	682.3	685.2
8A	Y	15	178.9	5.1	0	102.4	15.7	4.4	702.3	706.7
8A	Y	30	187.4	3.2	0	99.7	15.8	5.3	738.3	743.6
8A	Y	45	184	6.2	0	100.5	16.5	4.4	747.1	751.5
8A	Y	60	201.8	3.6	0	103.6	16.9	3.7	786.9	790.6
8A	Y	75	170.3	7.7	0	87.6	16	6.9	712	718.9

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C18:0	C18:1n9t	C18:1n11	C18:1n9c	C18:1n7	C18:2n6t	C18:2n6c	C18:2n6
8A	Y	90	183.4	3.3	2.1	111	12	5.3	568.6	573.9
1B	N	0	223.9	3.9	0	125.4	13	5.4	788.5	793.9
1B	N	15	190.5	1.8	0	113.8	11.1	4.3	739.3	743.6
1B	N	30	200.1	8.4	0	133.7	14.7	9.4	770.8	780.2
1B	N	45	1.2	9.2	0	149.2	12.7	7.1	796.8	803.9
1B	N	60	184.7	0.7	0	103.9	8.9	5.5	672.5	678
1B	N	75	185.3	1.3	0	125.9	11.3	18.2	727.7	745.9
1B	N	90	214.7	6.5	4.6	124.8	13.2	5	837.6	842.6
2B	N	0	214.9	7.1	0	169.5	14.8	6.1	814	820.1
2B	N	15	192.4	8.2	0	150.8	11.1	4.7	722.1	726.8
2B	N	30	197.2	4.2	0	158.6	12	3.8	738.1	741.9
2B	N	45	217.5	4	0	183.2	12.2	6	783.9	789.9
2B	N	60	195.8	14.7	0	145.7	10.7	6.4	717.4	723.8
2B	N	75	203.2	5.6	0	154.3	12.9	1	775.1	776.1
2B	N	90	197.6	1.8	3.4	132.5	11.5	6.3	691.6	697.9
3B	N	0	204.6	8	0	138.9	13.8	4	840.2	844.2
3B	N	15	173.3	8.1	0	99.9	10.4	4	713.3	717.3
3B	N	30	173.9	8.2	0	114	10.8	4.3	730.6	734.9
3B	N	45	157.5	3.6	0	113.4	8.8	3.9	653.5	657.4
3B	N	60	151.9	4.8	0	98.1	9.6	0.6	615.9	616.5
3B	N	75	177.9	5.7	0	118.6	11.4	4.8	765.3	770.1
3B	N	90	190	4.6	0	96.1	12.4	2.1	671.8	673.9
5B	N	0	220	6.2	0	115.4	13.6	2.3	766.5	768.8
5B	N	15	173.7	6.5	0	117.3	9.8	5.8	572.2	578
5B	N	30	160.4	4.7	0	116.2	8.3	0.2	576.3	576.5
5B	N	45	174.6	6.5	0	135.4	10.7	4	577.9	581.9

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C18:0	C18:1n9t	C18:1n11	C18:1n9c	C18:1n7	C18:2n6t	C18:2n6c	C18:2n6
5B	N	60	174.6	6.5	0	135.4	10.7	4	577.9	581.9
5B	N	75	159.2	6.1	0	110.3	10.9	5.2	562.4	567.6
5B	N	90	186	3.5	0	106.1	9.7	3.9	653.8	657.7
6B	N	0	213.3	10.6	0	155.2	12.8	5.1	670.6	675.7
6B	N	15	181.4	6.1	0	143.2	10.8	4.4	675.4	679.8
6B	N	30	215.9	4.1	0	158.3	13.3	5.3	689.4	694.7
6B	N	45	179	2.7	0	141.6	10.9	1	629.2	630.2
6B	N	60	201.5	1.6	0	169.5	12.6	1.6	684	685.6
6B	N	75	182.4	5.9	0	135.7	12.2	6.3	672.5	678.8
6B	N	90	185.8	3.6	0	149.4	13.2	7.9	705.2	713.1
7B	N	0	200.9	0.6	0	121.2	12	6.7	745.7	752.4
7B	N	15	176.2	4.2	0	125	10.1	3.5	721.9	725.4
7B	N	30	240.8	2.5	0	196.1	13.8	6	893.7	899.7
7B	N	45	228.1	6.3	0	147.4	11.9	6	880.8	886.8
7B	N	60	228.6	9.9	0	139.5	12.4	5.9	906.7	912.6
7B	N	75	269	6	0	167.4	18.1	18.8	897.4	916.2
7B	N	90	226.2	4.2	0	123.1	13.7	7	872.3	879.3
8B	N	0	385.8	11.4	0	534.1	41.7	3.4	1529	1532.4
8B	N	15	237.7	7.1	0	174.6	19	5.7	936.2	941.9
8B	N	30	258.5	12	0	185.6	18.8	5.4	1016.1	1021.5
8B	N	45	260.1	8.1	0	164.1	17.5	5.9	963	968.9
8B	N	60	211.6	9.3	0	134.9	14.5	1.2	816.5	817.7
8B	N	75	221.1	7.8	0	155.7	17.5	6.3	882.2	888.5
8B	N	90	190.8	3.3	0	142.7	16	1.2	837	838.2

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C18:3n6	C18:3n3	C20:3n6	C20:4n6	C20:5n3	C22:5n3	C22:6n3
1A	Y	0	5.4	42.5	4.9	0.6	3.3	5.4	3.6
1A	Y	15	7.2	8.9	4.9	3.7	3.2	4	15.2
1A	Y	30	7.2	8.9	4.9	3.7	3.2	4	15.2
1A	Y	45	3.4	9.8	5.8	4.1	0.9	0.9	15.4
1A	Y	60	3.6	10.5	4.7	2.4	2.4	6.7	11.9
1A	Y	75	3.1	12.8	5.1	5.8	3.5	1.3	11.6
1A	Y	90	0.8	8.5	7.1	4.7	1.3	3.6	1.6
2A	Y	0	7.4	90.4	5.1	0.9	2.5	2.8	5
2A	Y	15	4.2	9.5	4.6	4	0	2	6.5
2A	Y	30	6.4	7.4	4.5	2.1	1.2	2.5	7.5
2A	Y	45	4.4	8.5	7	2	1.3	3.5	13.9
2A	Y	60	6.2	9	5.1	3.7	0.4	3.3	6.8
2A	Y	75	4.2	11.7	8.4	2.3	1.1	3.2	2
2A	Y	90	1.3	17.8	6.7	12.6	1.1	5.7	5.9
3A	Y	0	3.6	23.9	3.7	3.7	2.8	9.2	3.1
3A	Y	15	8.7	15	6	4	1.4	1.1	7.2
3A	Y	30	4.5	13.4	5.5	2.9	1.4	1.6	13.9
3A	Y	45	1.3	13.1	6.3	2.4	1.1	1.7	0.4
3A	Y	60	4.8	9.2	6.2	5.2	1.5	2.4	2.2
3A	Y	75	2.2	7.2	4.3	4.2	0	3.8	1.4
3A	Y	90	2.7	10.8	6.6	2.7	1	3.4	7.5
4A	Y	0	2.5	35.4	7.1	0	1.8	3.6	5.7
4A	Y	15	4.4	13.3	5.5	3.5	0	2.8	7.7
4A	Y	30	0.6	19.3	5.6	4.5	1.5	6.4	6.1
4A	Y	45	1.1	18.4	5.8	3.2	2.1	4.2	9.5
4A	Y	60	2.6	8.8	6.6	2	0	3.7	7.2
4A	Y	75	3.4	10.9	7.9	2.1	0	3.7	6.9
4A	Y	90	5.2	18.6	8.3	0.5	2	3.7	8.6

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C18:3n6	C18:3n3	C20:3n6	C20:4n6	C20:5n3	C22:5n3	C22:6n3
5A	Y	0	7.6	26.3	4.3	0	0.4	0.9	4
5A	Y	15	6	13.6	5.3	2.2	2.3	2	8.7
5A	Y	30	4.8	12	4.9	2.8	0	1.7	9.2
5A	Y	45	1.5	12.6	5.5	3.3	1.3	2.8	17.9
5A	Y	60	4.6	8.7	5.5	4.2	1.4	1.9	9.1
5A	Y	75	2.9	9.7	6	1.1	2.1	2.5	0.9
5A	Y	90	2.7	10	6	1.8	0	3.6	10.6
6A	Y	0	2.7	24.5	5.9	4.5	0.8	5	6.4
6A	Y	15	4.2	11.7	7.3	3.2	0	4	9.9
6A	Y	30	5.8	17.4	7.1	2.5	1.4	3.7	7.7
6A	Y	45	3.1	18.9	9	5.5	1.2	0	5.5
6A	Y	60	6.5	17.3	6.6	2.8	1.3	3.4	6.8
6A	Y	75	2.4	11.2	6.3	1.8	1.4	3.8	8.1
6A	Y	90	4.8	13.8	4.7	2.4	2.3	0.7	6.6
7A	Y	0	6.1	21.9	9.2	4.1	1.7	3.5	8.6
7A	Y	15	6.4	25.3	5.9	3.8	0.9	0.6	9.3
7A	Y	30	2.4	12	5.1	4.1	0	1.8	10
7A	Y	45	1.5	13.3	5.2	3.9	0	1.9	9.6
7A	Y	60	5.2	19	5.1	2.4	7.6	1.7	8.2
7A	Y	75	3.5	15.1	5.3	0.8	0	2.9	21.9
7A	Y	90	3.5	11.6	9.1	5	0	3.6	13.6
8A	Y	0	6	27.8	6.8	1.5	0	3	4
8A	Y	15	4	13.1	6	4.3	1.1	1.5	12.7
8A	Y	30	6.5	14.3	5.8	2.8	3.5	5.1	13.1
8A	Y	45	3.2	10.2	7.8	3	2.5	1	8.1
8A	Y	60	0.4	13	10.3	4.2	0.4	3.4	11
8A	Y	75	1.5	13.2	9.4	6.2	0	0	10.7
8A	Y	90	2.4	11.3	7.3	4.2	0.6	3.7	8.3

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C18:3n6	C18:3n3	C20:3n6	C20:4n6	C20:5n3	C22:5n3	C22:6n3
1B	N	0	6.6	25.8	9.4	0.5	3.3	1	4.9
1B	N	15	6.1	14.3	7.1	2.4	2.9	2.5	5
1B	N	30	3.3	10.4	4.6	2	5.9	3.8	6
1B	N	45	3.2	15.2	8	2.4	0.4	3.5	7.1
1B	N	60	1.7	10.9	6.5	1.3	1.2	2.9	3.8
1B	N	75	3.8	6.9	7.8	0	2.8	3.7	5.5
1B	N	90	2.6	22.5	6.2	4.5	1	2.5	1.6
2B	N	0	2.9	30.9	8.5	3.5	0.8	0.7	8.9
2B	N	15	3.1	28.7	7.1	1.2	4.2	1.7	5.8
2B	N	30	6.2	16.8	4.4	3.3	1.2	2.1	0.2
2B	N	45	2.2	17.9	7.4	4	1.5	1.1	1.3
2B	N	60	4.6	19.2	9.7	0.7	4.2	2	3.4
2B	N	75	3.9	16.9	9.9	0	0	2.1	0
2B	N	90	3.2	17.9	9	1.6	1.7	2.6	0.7
3B	N	0	5.6	19.3	4.1	0.9	4.4	1.2	4.6
3B	N	15	2.3	21	8	1.3	2.7	1.6	4.3
3B	N	30	3.7	12.5	9.1	1.4	0	2	4.8
3B	N	45	1.6	15.7	6	0.1	1.1	2.3	4.4
3B	N	60	2	16	5.1	2.2	2.9	3.4	3.4
3B	N	75	2.7	9.2	5.3	3.7	2.2	3.6	1.6
3B	N	90	3.3	19	15.7	1.1	0	2.9	3.2
5B	N	0	6.2	14.2	4.8	0.5	2.3	5.9	5.8
5B	N	15	4.3	17.1	6.7	0	3.9	5	6.1
5B	N	30	4.9	19	3.1	0	0	3.9	2.7
5B	N	45	0	18.2	5.1	3.3	3.1	3.6	4
5B	N	60	1.5	18.7	3.7	0.7	3.1	0.2	1
5B	N	75	1.8	12.9	4.3	0	1.6	3.7	3
5B	N	90	1.5	13.2	5.9	1.2	4.5	4	2.9

APPENDIX 7 CONTINUED.

Horse	Trt	Day	C18:3n6	C18:3n3	C20:3n6	C20:4n6	C20:5n3	C22:5n3	C22:6n3
6B	N	0	1.8	44.5	8.5	3.5	4.5	3.8	3.1
6B	N	15	5.7	24.8	8.1	3.5	2.9	3.4	2.3
6B	N	30	0.7	15.4	1.1	2.6	3.1	2.1	4.7
6B	N	45	5	16.3	6.2	2.6	0	5	4.8
6B	N	60	9.3	21.6	6.8	2.3	0	4.7	2.8
6B	N	75	3.1	10.1	7.3	3.1	1.7	3.8	2.5
6B	N	90	2.5	14	9.8	0	2.1	3.5	2.9
7B	N	0	2.4	28.8	5.5	1.2	0.6	4	3.7
7B	N	15	4.7	14.4	7.4	1	0.6	0.2	4.8
7B	N	30	3.9	29.4	4.9	1.4	0	3.6	1.1
7B	N	45	4.2	15.4	5	2.8	2.5	3.1	3.3
7B	N	60	5.5	18.9	6.2	1.2	1.3	0.6	2.8
7B	N	75	6.7	17	4	3.7	2.8	2.3	2
7B	N	90	16.7	21.7	6.4	2.5	3.2	2.1	2.3
8B	N	0	14	90.3	8.5	2	2.8	3.9	5.6
8B	N	15	4.5	16.2	5.6	0.1	3.8	3.4	3.6
8B	N	30	3.4	20	4.1	0	3.4	0	4.4
8B	N	45	4.2	17.6	4.7	1.7	5.1	3.4	3.8
8B	N	60	1.1	13.2	3.3	1.1	2.3	3.9	2.4
8B	N	75	3.1	13.9	6	1.3	2.1	0.7	2.3
8B	N	90	9	17	5.7	0.5	4.4	0.9	0.4

APPENDIX 8. DAILY GROSS ENERGY (Mcal) OF EACH HORSE.

Horse	Weight (lbs)	T	Grain intake/day (g)	Grain (Mcal))	Supp A (Mcal)	Supp B (Mcal)	Hay (Mcal)	Total(Mcal)
1A	816	Y	2854.55	11.55	1.52	1.54	25.18	39.79
2A	838	Y	2945.45	11.92	1.52	1.54	25.18	40.16
3A	1108	Y	4172.73	16.88	1.52	1.54	25.18	45.12
4A	1171	Y	4463.64	18.06	1.52	1.54	25.18	46.30
5A	818	Y	2854.55	11.55	1.52	1.54	25.18	39.79
6A	1092	Y	4100.00	16.59	1.52	1.54	25.18	44.83
7A	920	Y	3318.18	13.43	1.52	1.54	25.18	41.67
8A	1164	Y	2727.27	11.03	1.52	1.54	25.18	39.27
1B	1102	N	5009.09	20.27	-	-	25.18	45.45
2B	1160	N	4090.91	16.55	-	-	25.18	46.51
3B	1163	N	5290.91	21.41	-	-	25.18	46.59
4B	1120	N	5090.91	20.60	-	-	25.18	45.78
5B	976	N	4436.36	17.95	-	-	25.18	43.13
6B	1088	N	4945.45	20.01	-	-	25.18	45.19
7B	884	N	4018.18	16.26	-	-	25.18	41.44
8B	998	N	4536.36	18.35	-	-	25.18	43.53

APPENDIX 9. MEAN PLASMA PGE_2 BY TREATMENT AND DAY.

	Concentrations (pg/ml)		Normalized values	
Day	Control	Treatment	Control	Treatment
0	2191 ± 1381	2135 ± 1378	0	0
15	2436 ±1248	1689 ± 700	245 ± 609	-446 ± 771
30	2712 ± 1539	1721 ± 812	521 ± 227	-414 ± 729
45	2680 ± 1387	1506± 833	448 ± 695	-628 ± 566
60	2764 ± 1363	1635 ± 928	573 ± 703	-500 ± 456
75	2574 ± 1130	1382 ± 530	383 ± 987	-753 ± 1071
90	3765 ± 1592	1939 ± 875	1573 ± 1311	-196 ±754
Total	2732 ± 494	1715 ± 319	540 ± 278	-420 ± 247

APPENDIX 10. MEAN PLASMA FIBRINOGEN BY TREATMENT AND DAY.

	Concentrations (mg/dl)		Normalized	
Day	Control	Treatment	Control	Treatment
0	398 ± 15	466 ± 30	0	0
15	426 ± 43	502 ± 33	28 ± 46	35 ± 36
30	420 ± 17	437 ± 39	22 ± 25	-29 + 36
45	375 ± 20	396 ± 30	-22 ± 26	-70 ± 27
60	365 ± 28	397 ± 17	-48 ± 19	-87 ± 34
75	371 ± 17	406 ± 26	-42 ± 15	-78 ± 39
90	387 ± 19	418 ± 32	-26 ± 24	-66 ± 44
Total	394 ± 10	433 ± 12	-10 ± 10	-40 ± 13

APPENDIX 11. MEAN SYNOVIAL FLUID WBC BY TREATMENT AND DAY.

	Concentrations (cells/ml)		Normalized	
Day	Control	Treatment	Control	Treatment
0	336 ± 80	382 ± 96	0	0
30	888 ± 698	172 + 75	582 ± 661	-199 ± 149
60	200 ± 67	66 ± 11	-136 ± 61	-316 ± 100
90	343 ± 136	72 ± 9	5 ± 84	-388 ± 124
Total	420 ± 155	181 ± 38	90 ± 144	-214 ± 55

APPENDIX 12. PLASMA PGE2 AND FIBRINOGEN FOR ALL HORSES.

Horse	Day	Trt (Y/N)	PGE_2 (pg/ml)	Fibrinogen (mg/dl)
1A	0	Y	487	569
1A	15	Y	1074	579
1A	30	Y	235	536
1A	45	Y	111	477
1A	60	Y	403	393
1A	75	Y	476	402
1A	90	Y	600	439
2A	0	Y	1914	374
2A	15	Y	2071	523
2A	30	Y	2267	427
2A	45	Y	1407	384
2A	60	Y	1211	433
2A	75	Y	1497	446
2A	90	Y	1885	480
3A	0	Y	10301	589
3A	15	Y	*	485
3A	30	Y	5885	451
3A	45	Y	6372	439
3A	60	Y	7150	410
3A	75	Y	3069	445
3A	90	Y	6309	416
4A	0	Y	952	492
4A	15	Y	2350	383
4A	30	Y	2881	273
4A	45	Y	1388	299
4A	60	Y	1119	331
4A	75	Y	3194	275
4A	90	Y	4760	301
5A	0	Y	201	481
5A	15	Y	127	635
5A	30	Y	*	500
5A	45	Y	265	482
5A	60	Y	399	439
5A	75	Y	*	470
5A	90	Y	314	533
6A	0	Y	9880	428
6A	15	Y	*	500
6A	30	Y	20989	358
6A	45	Y	13759	326
6A	60	Y	*	338
6A	75	Y	12194	348
6A	90	Y	11717	308

APPENDIX 12 CONTINUED.

Horse	Day	Trt (Y/N)	PGE_2 (pg/ml)	Fibrinogen (mg/dl)
7A	0	Y	873	461
7A	15	Y	626	555
7A	30	Y	504	456
7A	45	Y	507	485
7A	60	Y	802	440
7A	75	Y	735	456
7A	90	Y	*	450
8A	0	Y	*	341
8A	15	Y	171	361
8A	30	Y	215	358
8A	45	Y	495	282
8A	60	Y	361	334
8A	75	Y	286	312
8A	90	Y	*	329
1B	0	N	10258	380
1B	15	N	8348	352
1B	30	N	11308	312
1B	45	N	8977	409
1B	60	N	8909	360
1B	75	N	*	367
1B	90	N	9183	397
2B	0	N	*	471
2B	15	N	392	349
2B	30	N	390	334
2B	45	N	411	300
2B	60	N	593	412
2B	75	N	358	372
2B	90	N	319	333
3B	0	N	99	439
3B	15	N	253	507
3B	30	N	53	498
3B	45	N	68	456
3B	60	N	311	466
3B	75	N	171	448
3B	90	N	31	454
4B	0	N	*	372
4B	15	N	*	690
4B	30	N	*	507
4B	45	N	3461	393
4B	60	N	*	731
4B	75	N	*	*
4B	90	N	*	*

APPENDIX 12 CONTINUED.

Horse	Day	Trt (Y/N)	PGE_2 (pg/ml)	Fibrinogen (mg/dl)
5B	0	N	528	387
5B	15	N	754	327
5B	30	N	830	329
5B	45	N	867	365
5B	60	N	328	298
5B	75	N	5052	357
5B	90	N	9039	371
6B	0	N	2457	380
6B	15	N	5935	360
6B	30	N	4418	380
6B	45	N	6938	285
6B	60	N	7032	277
6B	75	N	9060	320
6B	90	N	5202	340
7B	0	N	1451	425
7B	15	N	1115	450
7B	30	N	1660	377
7B	45	N	836	414
7B	60	N	1253	378
7B	75	N	742	366
7B	90	N	*	421
8B	0	N	*	331
8B	15	N	259	376
8B	30	N	328	412
8B	45	N	328	381
8B	60	N	521	401
8B	75	N	382	358
8B	90	N	128	344

APPENDIX 13. SYNOVIAL FLUID WBC FOR ALL HORSES.

Horse	Joint	Day	Trt (Y/N)	Synovial Fluid WBC (cells/ml)
1A	Upper L Knee	0	Y	875
1A	Upper L Knee	30	Y	88
1A	Upper L Knee	60	Y	47
1A	Upper L Knee	90	Y	80
1A	Lower L Knee	0	Y	917
1A	Lower L Knee	30	Y	70
1A	Lower L Knee	60	Y	21
1A	Lower L Knee	90	Y	40
2A	Upper R Knee	0	Y	291
2A	Upper R Knee	30	Y	189
2A	Upper R Knee	60	Y	142
2A	Upper R Knee	90	Y	107
2A	Lower R Knee	0	Y	229
2A	Lower R Knee	30	Y	299
2A	Lower R Knee	60	Y	266
2A	Lower R Knee	90	Y	224
3A	Upper L Knee	0	Y	92
3A	Upper L Knee	30	Y	820
3A	Upper L Knee	60	Y	41
3A	Upper L Knee	90	Y	89
3A	Lower L Knee	0	Y	732
3A	Lower L Knee	30	Y	76
3A	Lower L Knee	60	Y	43
3A	Lower L Knee	90	Y	47
4A	Upper R Knee	0	Y	129
4A	Upper R Knee	30	Y	249
4A	Upper R Knee	60	Y	59
4A	Upper R Knee	90	Y	56
4A	Lower R Knee	0	Y	78
4A	Lower R Knee	30	Y	29
4A	Lower R Knee	60	Y	51
4A	Lower R Knee	90	Y	137
5A	Left Hind Fetlock	0	Y	488
5A	Left Hind Fetlock	30	Y	2250
5A	Left Hind Fetlock	60	Y	106
5A	Left Hind Fetlock	90	Y	105
6A	Upper R Knee	0	Y	193
6A	Upper R Knee	30	Y	89
6A	Upper R Knee	60	Y	122
6A	Upper R Knee	90	Y	74

APPENDIX 13 CONTINUED.

Horse	Joint	Day	Trt (Y/N)	Synovial Fluid WBC (cells/ml)
6A	Lower L Knee	0	Y	106
6A	Lower L Knee	30	Y	48
6A	Lower L Knee	60	Y	38
6A	Lower L Knee	90	Y	41
7A	Left Hind Fetlock	0	Y	311
7A	Left Hind Fetlock	30	Y	68
7A	Left Hind Fetlock	60	Y	64
7A	Left Hind Fetlock	90	Y	94000
8A	Upper L Hock	0	Y	57
8A	Upper L Hock	30	Y	83
8A	Upper L Hock	60	Y	37
8A	Upper L Hock	90	Y	43
8A	Upper R Hock	0	Y	41
8A	Upper R Hock	30	Y	27
8A	Upper R Hock	60	Y	*
8A	Upper R Hock	90	Y	49
1B	Upper R Knee	0	N	602
1B	Upper R Knee	30	N	2500
1B	Upper R Knee	60	N	316
1B	Upper R Knee	90	N	1160
1B	Lower R Knee	0	N	814
1B	Lower R Knee	30	N	542
1B	Lower R Knee	60	N	593
1B	Lower R Knee	90	N	678
2B	Upper L Knee	0	N	162
2B	Upper L Knee	30	N	500
2B	Upper L Knee	60	N	93
2B	Upper L Knee	90	N	93
2B	Lower L Knee	0	N	567
2B	Lower L Knee	30	N	5750
2B	Lower L Knee	60	N	107
2B	Lower L Knee	90	N	740
3B	Upper R Knee	0	N	111
3B	Upper R Knee	30	N	41
3B	Upper R Knee	60	N	31
3B	Upper R Knee	90	N	98
3B	Lower R Knee	0	N	102
3B	Lower R Knee	30	N	19
3B	Lower R Knee	60	N	32
3B	Lower R Knee	90	N	43

APPENDIX 13 CONTINUED.

Horse	Joint	Day	Trt (Y/N)	Synovial Fluid WBC (cells/ml)
4B	Upper L Knee	0	N	184
4B	Upper L Knee	30	N	116
4B	Upper L Knee	60	N	*
4B	Upper L Knee	90	N	*
4B	Lower L Knee	0	N	105
4B	Lower L Knee	30	N	*
4B	Lower L Knee	60	N	*
4B	Lower L Knee	90	N	*
5B	Left Hind Fetlock	0	N	305
5B	Left Hind Fetlock	30	N	85
5B	Left Hind Fetlock	60	N	146
5B	Left Hind Fetlock	90	N	93
6B	Left Front Fetlock	0	N	316
6B	Left Front Fetlock	30	N	*
6B	Left Front Fetlock	60	N	550
6B	Left Front Fetlock	90	N	*
6B	Right Front Fetlock	0	N	355
6B	Right Front Fetlock	30	N	46
6B	Right Front Fetlock	60	N	63
6B	Right Front Fetlock	90	N	48
7B	Upper R Knee	0	N	132
7B	Upper R Knee	30	N	121
7B	Upper R Knee	60	N	71
7B	Upper R Knee	90	N	142
7B	Lower R Knee	0	N	875
7B	Lower R Knee	30	N	122
7B	Lower R Knee	60	N	89
7B	Lower R Knee	90	N	109
8B	Right Stifle	0	N	174
8B	Right Stifle	30	N	341
8B	Right Stifle	60	N	481
8B	Right Stifle	90	N	161

APPENDIX 14. ANOVA FOR MEAN FIBRINOGEN CONCENTRATION (mg/dl).

Source	Partial SS	df	MS	F	Prob > F
Model	139193.32	13	10707.18	1.76	0.062
Treatment	40864.72	1	40864.72	6.72	0.011
Day	85903.32	6	14317.22	2.36	0.037
Treatment by day	12626.16	6	2104.36	0.35	0.91
Residual	540918.41	89	6.077.73		
Total	680111.72	102	6667.76		

APPENDIX 15. ANOVA FOR NORMALIZED MEAN FIBRINOGEN CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	161290.46	13	12406.95	1.79	0.057
Treatment	22398.53	1	22398.53	3.22	0.076
Day	122623.93	6	20437.32	2.94	0.011
Treatment by day	13056.63	6	2176.1	0.31	0.928
Residual	618575.51	89	6950.28		
Total	779865.98	102	6950.28		

APPENDIX 16. ANOVA FOR MEAN WBC CONCENTRATIONS (cells/ml).

Source	Partial SS	df	MS	F	Prob > F
Model	4144465.06	7	592066.43	1.31	0.25
Treatment	1367337.35	1	1367337.35	3.02	0.08
Day	1739802.73	3	579934.24	1.28	0.28
Treatment by day	1495548.92	3	498516.3	1.1	0.35
Residual	31211361.3	69	452338.56		
Total	35355826.3	76	465208.24		

APPENDIX 17. ANOVA FOR NORMALIZED MEAN WBC CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	5246782.94	7	749540.42	1.76	0.1
Treatment	2179617.2	1	2179617.2	5.13	0.02
Day	2070631.73	3	690210.57	1.62	0.19
Treatment by day	1610193.58	3	536731.19	1.26	0.29
Residual	29311984.5	69	424811.37		
Total	34558767.5	76	454720.62		

APPENDIX 18. ANOVA FOR MEAN PGE_2 CONCENTRATIONS (pg/ml).

Source	Partial SS	df	MS	F	Prob > F
Model	38381057.7	13	2952389.06	0.31	0.98
Treatment	25331233.4	1	25331233.4	2.65	0.1
Day	7030586.97	6	1171764.5	0.12	0.99
Treatment by day	6019237.4	6	1003206.23	0.11	0.99
Residual	802102692	84	9548841.57		
Total	840483750	97	8664780.93		

APPENDIX 19. ANOVA FOR NORMALIZED MEAN PGE_2 CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	35665711.3	13	2743516.25	0.73	0.72
Treatment	22615886.9	1	22615886.9	6.05	0.01
Day	7030586.97	6	1171764.5	0.31	0.92
Treatment by day	6019237.4	6	1003206.23	0.27	0.95
Residual	314001263	84	3738110.28		
Total	349666974	97	3604814.17		

APPENDIX 20. ANOVA FOR ENERGY INTAKE BY GROUP.

Source	Partial SS	df	MS	F	Prob > F
Model	15.82	1	15.82	2.67	0.12
Treatment	15.82	1	15.82	2.67	0.12
Residual	82.65	14	5.91		
Total	98.65	15	6.57		

APPENDIX 21. ANOVA FOR MEAN LINOLEIC ACID (C18:2) INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	2511.30	1	2511.30	11.01	0.0056
Treatment	2511.30	1	2511.30	11.01	0.0056
Residual	2965.95	13	22.15		
Total	5477.25	148	391.23		

APPENDIX 22. ANOVA FOR MEAN GAMMA LINOLEIC ACID (C18:3n6) INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	0.105	1	0.105	275	0.0000
Treatment	0.105	1	0.105	275	0.0000
Residual	0.005	13	0.0004		
Total	0.11	14	0.008		

APPENDIX 23. ANOVA FOR MEAN ALPHA-LINOLENIC ACID (C18:3n3) INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	5.55	1	5.55	3.59	0.0000
Treatment	5.55	1	5.55	3.59	0.0000
Residual	20.09	13	1.55		
Total	25.65	14	1.83		

APPENDIX 24. ANOVA FOR MEAN C20:3n6 INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	0.05	1	0.054	1261	0.0000
Treatment	0.05	1	0.054	1261	0.0000
Residual	0.0006	13	0.00004		
Total	0.055	14	0.004		

APPENDIX 25. ANOVA FOR MEAN ARACHIDONIC ACID (C20:4n6) INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	0.50	1	0.50	3131	0.0000
Treatment	0.50	1	0.50	3131	0.0000
Residual	0.002	13	0.0002		
Total	.050	14	0.36		

APPENDIX 26. ANOVA FOR MEAN EICOSAPENTAENOIC ACID (C20:5n3) INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	0.0008	1	0.0008	0.42	0.53
Treatment	0.0008	1	0.0008	0.42	0.53
Residual	0.025	13	0.002		
Total	0.026	14	0.002		

APPENDIX 27. ANOVA FOR MEAN DOCOSAPENTAENOIC ACID (C22:5n3) INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	292.5	1	292.5	88064	0.0000
Treatment	292.5	1	292.5	88064	0.0000
Residual	0.04	13	0.003		
Total	292.5	14	20.89		

APPENDIX 28. ANOVA FOR MEAN DOCOSAHEXAENOIC ACID (C22:6n3) INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	0.0003	1	0.0003	7.32	0.02
Treatment	0.0003	1	0.0003	7.32	0.02
Residual	0.0006	13	0.00004		
Total	0.0009	14	0.00006		

APPENDIX 29. ANOVA FOR MEAN n3 FATTY ACID INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	217.2	1	217.2	119.5	0.0000
Treatment	217.2	1	217.2	119.5	0.0000
Residual	23.6	13	1.82		
Total	240.9	14	17.21		

APPENDIX 30. ANOVA FOR MEAN n6 FATTY ACID INTAKE.

Source	Partial SS	df	MS	F	Prob > F
Model	2386	1	2386	10.41	0.0066
Treatment	2386	1	2386	10.41	0.0066
Residual	2980	13	229.3		
Total	5367	14	383.4		

APPENDIX 31. ANOVA FOR MEAN LINOLEIC ACID (C18:2n6) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	797847	13	61372	2.45	0.007
Treatment	258212	1	258212	10.3	0.0018
Day	375453	6	62575	2.5	0.03
Treatment by day	133739	6	22289	0.89	0.51
Residual	2281332	91	25069		
Total	3079179	104	29069		

APPENDIX 32. ANOVA FOR NORMALIZED MEAN LINOLEIC ACID (C18:2n6) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	4949021	13	380693	69.6	0.0000
Treatment	27895	1	27895	5.1	0.03
Day	4875873	6	812645	148.5	0.0000
Treatment by day	5313	6	885	0.2	1.0
Residual	498132	91	5473		
Total	5447154	104	52376		

APPENDIX 33. ANOVA FOR MEAN GAMMA-LINOLENIC ACID (C18:3n6) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	122	13	9.39	1.57	0.11
Treatment	1.04	1	1.04	0.17	0.70
Day	79.9	6	13.3	2.2	0.05
Treatment by day	39.1	6	6.52	1.09	0.37
Residual	544	91	6.0		
Total	666	104	6.4		

APPENDIX 34. ANOVA FOR NORMALIZED MEAN GAMMA-LINOLENIC ACID (C18:3n6) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	3442795	13	264830	218	0.0000
Treatment	1096	1	1096	0.9	0.34
Day	3400710	6	566785	467.9	0.0000
Treatment by day	6252	6	1042	0.9	0.53
Residual	110235	91	1211		
Total	3553030	104	34163		

APPENDIX 35. ANOVA FOR MEAN ALPHA-LINOLENIC ACID (C18:3n3) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	6724	13	517	.3	0.0000
Treatment	319	1	319	3.3	0.07
Day	6186	6	1031	105	0.0000
Treatment by day	119	6	19	0.2	0.97
Residual	8915	91	97		
Total	15639	104	150		

APPENDIX 36. ANOVA FOR NORMALIZED MEAN ALPHA-LINOLENIC ACID (C18:3n3) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	6787	13	522	1.0	0.5
Treatment	382	1	382	0.7	0.4
Day	6186	6	1031	1.9	0.09
Treatment by day	119	6	19.9	0.04	1.0
Residual	48914	91	537		
Total	55701	104	535		

APPENDIX 37. ANOVA FOR MEAN C20:3n6 CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	79.5	13	6.1	1.8	0.05
Treatment	2.15	1	2.2	0.6	0.43
Day	57.4	6	9.6	2.8	0.02
Treatment by day	23.2	6	3.9	1.1	0.35
Residual	310	91	3.4		
Total	389	104	3.7		

APPENDIX 38. ANOVA FOR NORMALIZED MEAN C20:3n6 CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	97.9	13	7.5	1.3	0.2
Treatment	20.3	1	20.3	3.6	0.06
Day	57.4	6	9.6	1.7	0.13
Treatment by day	23.2	6	3.9	0.7	0.66
Residual	509	91	5.6		
Total	606	104	5.8		

APPENDIX 39. ANOVA FOR MEAN ARACHIDONIC ACID (C20:4n6) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	94.2	13	7.2	2.64	0.004
Treatment	64.9	1	64.9	23.7	0.0000
Day	13.1	6	2.2	0.8	0.6
Treatment by day	14.9	6	2.5	0.9	0.50
Residual	249.9	91	2.7		
Total	344	104	3.3		

APPENDIX 40. ANOVA FOR NORMALIZED MEAN ARACHIDONIC ACID (C20:4n6) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	79.9	13	6.1	1.4	0.2
Treatment	50.7	1	507	11.3	0.001
Day	13.1	6	2.2	0.5	0.82
Treatment by day	14.9	6	2.5	0.6	0.77
Residual	408	91	4.5		
Total	488	104	4.7		

APPENDIX 41. ANOVA FOR MEAN EICOSAPENTAENOIC ACID (C20:5n3) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	35.4	13	2.7	1.2	0.3
Treatment	22.5	1	22.5	10.2	0.002
Day	6.0	6	1.0	0.5	0.8
Treatment by day	7.1	6	1.2	0.5	0.8
Residual	200	91	2.2		
Total	236	104	2.3		

APPENDIX 42. ANOVA FOR NORMALIZD MEAN EICOSAPENTAENOIC ACID (C20:5n3) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	46.9	13	3.6	1.5	0.13
Treatment	5.0	1	5.0	2.1	0.15
Day	26.1	6	4.4	1.8	0.10
Treatment by day	17.9	6	3.0	1.3	0.300
Residual	216	91	2.4		
Total	263	104	2.5		

APPENDIX 43. ANOVA FOR MEAN DOCOSAHEXAENOIC ACID (C22:6n3) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	843	13	94.9	6.0	0.0000
Treatment	325	1	625	57.8	0.0000
Day	91	6	15.2	1.4	0.22
Treatment by day	120	6	20.1	1.9	0.09
Residual	984	91	10.8		
Total	1828	104	17.6		

APPENDIX 44. ANOVA FOR NORMALIZED MEAN DOCOSAHEXAENOIC ACID (C22:6n3) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	890	13	68	5.4	0.0000
Treatment	671	1	671	52.6	0.0000
Day	91.4	6	15.2	1.2	0.32
Treatment by day	120	6	20	1.6	0.20
Residual	1162	91	12.8		
Total	2053	104	19.7		

APPENDIX 45. ANOVA FOR MEAN DOCOSAPENTAENOIC ACID (C22:5n3) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	33.1	13	2.5	1.0	0.4
Treatment	2.4	1	2.4	1.0	0.3
Day	12.5	6	2.1	0.9	0.5
Treatment by day	16.5	6	2.7	1.1	0.4
Residual	221	91	2.4		
Total	254	104	2.5		

APPENDIX 46. ANOVA FOR NORMALIZED MEAN DOCOSAPENTAENOIC ACID (C22:5n3) CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	54	13	4.2	0.7	0.75
Treatment	23	1	23.3	4.0	0.048
Day	12.5	6	2.1	0.4	0.90
Treatment by day	16	6	2.7	0.5	0.83
Residual	532	91	5.9		
Total	586	104	5.6		

APPENDIX 47. ANOVA FOR MEAN n6 CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	793975	13	61075	2.4	0.008
Treatment	252578	1	252578	10.0	0.002
Day	381190	6	63531	2.5	0.03
Treatment by day	130022	6	21670	0.9	0.53
Residual	2304276	91	25321		
Total	3098251	104	29790		

APPENDIX 48. ANOVA FOR NORMALIZED MEAN n6 CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	1282405	13	98646	1.4	0.20
Treatment	741009	1	741009	10.2	0.002
Day	381190	6	63531	0.9	0.52
Treatment by day	130022	6	21670	0.3	0.94
Residual	6612972	91	72670		
Total	7895377	104	75917		

APPENDIX 49. ANOVA FOR MEAN n3 CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	6663	13	512	4.6	0.0000
Treatment	15.4	1	15.4	0.1	0.71
Day	6551	6	1091	9.8	0.0000
Treatment by day	92.6	6	15.4	0.1	1.00
Residual	10157	91	111		
Total	16820	104	161		

APPENDIX 50. ANOVA FOR NORMALIZED MEAN n3 CONCENTRATIONS.

Source	Partial SS	df	MS	F	Prob > F
Model	6651	13	511	0.9	0.55
Treatment	3.8	1	3.8	0.01	0.94
Day	6551	6	1091	1.9	0.08
Treatment by day	92.7	6	15.4	0.03	1.0
Residual	51244	91	563		
Total	57896	104	556		

APPENDIX 50. ANOVA FOR NORMALIZED WEIGHT DISTRIBUTION (FORCE PLATES).

Source	Partial SS	df	MS	F	Prob > F
Model	126	7	18.1	1.44	0.25
Treatment	32.7	1	32.7	2.61	0.12
Day	40	3	13.5	1.08	0.38
Treatment by day	60	3	20.1	1.61	0.22
Residual	225	18	12.6		
Total	351	25	14.1		

VITA

Name:	Denise Rae Manhart
Address:	Department of Animal Science, Equine Science Section, 249 Kleberg Center, 2471 TAMU, College Station, TX 77843-2471
Email Address:	denisemanhart@hotmail.com
Education:	B.S., Animal Science, Oregon Station University, 2005 M.S., Animal Science, Texas A&M University, 2007