Omega-3 Fatty Acid Supplementation In The Management Of Chronic Lower Airway Inflammatory Conditions In Horses

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By Nora Nogradi

Entitled
OMEGA-3 FATTY ACID SUPPLEMENTATION IN THE MANAGEMENT OF CHRONIC LOWER AIRWAY INFLAMMATORY DISEASES IN HORSES

For the degree of Master of Science

Is approved by the final examining committee:

Dr. Laurent L. Couetil
Chair

Dr. Joanne Messick

Dr. John Burgess

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Approved by Major Professor(s): Dr. Laurent L. Couetil

Approved by: Dr. Peter Constable 07/05/2013

Head of the Graduate Program Date
OMEGA-3 FATTY ACID SUPPLEMENTATION IN THE MANAGEMENT OF CHRONIC LOWER AIRWAY INFLAMMATORY CONDITIONS IN HORSES

A Thesis
Submitted to the Faculty
of
Purdue University
by
Nora Nogradi

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

August 2013
Purdue University
West Lafayette, Indiana
ACKNOWLEDGEMENTS

I would like to express my special thanks to Novus Nutrition Brands, LLC and Purina Mills, LLC for providing funding for this graduate research project. Without their support neither my graduate program, nor the experiments could have been carried out.

I am very thankful to my Mentor and Major Professor Dr. Laurent L. Couëtil for accepting me as a graduate student, guiding me through the program, helping me setting up the research plan, chairing my thesis committee and participating in the experiments. This work could have not been carried out without his help.

I acknowledge Dr. John Burgess and Dr. Joanne Messick, as members of my Thesis Committee, who provided specialized support to carry out the experiments.

I would like to express special thanks to Donna Griffey, who actively participated in the experiments on a daily basis, provided invaluable technical help and support. I would like to thank Dr. Kathleen Ivester and Dr. Munsiff Kamarudin; fellow graduate students who were a great help carrying out the experiments, as well as all the senior veterinary students at the Large Animal Clinic who helped with taking care of the horses during their clinical rotations.

Last but not least I would like to thank all the referring veterinarians, horses and their owners who participated in the project.
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ABSTRACT

Nora Nogradi. M.S., Purdue University August, 2013. Omega-3 fatty acid supplementation in the management of chronic lower airway inflammatory conditions in horses. Major Professor: Laurent L. Couëtil.

Chronic lower airway inflammatory diseases, such as recurrent airway obstruction (RAO) and inflammatory airway disease (IAD) are common in horses. RAO and IAD present many similarities with asthma in people. Based on studies in human asthma, omega – 3 polyunsaturated fatty acid (PUFA) supplementation has been proposed to improve clinical signs in horses with chronic lower airway inflammatory disease. The purpose of this study was to evaluate the clinical efficacy of an omega-3 PUFA containing equine feed supplement (Aleira™) in horses with RAO and IAD. The study consisted of two separate experiments. First, a pilot study was performed in 8 research horses owned by Purdue University, to find the minimal effective dose that is able to alter the composition of phospholipid classes in plasma. Once the minimal effective dose was identified, a randomized, double blind, placebo controlled clinical trial was performed on client-owned horses with a history of chronic respiratory disease (>1 month). Eligible horses were randomly assigned into one of 3 treatment groups fed daily for 2 months with: 30 g of the supplement, 60 g of the supplement or 30 g of placebo. Additionally, all horses in
the study were maintained on a complete pelleted diet for the duration of the study with no exposure to hay. Multiple clinical and clinicopathologic parameters were measured, and lung function testing was performed before and after the 8-week supplementation period. Data was analyzed using Wilcoxon matched pairs test. Data were expressed as median [25th-75th percentiles] and P<0.05 was considered significant. Clinical improvement was noted in all horses, however the group receiving the supplement had a greater improvement in the veterinarian assigned clinical score and the owner assigned visual analog score, when compared to placebo (P<0.05). Our results indicate that daily feeding with 30 g or 60 g of omega-3 PUFA feed supplement for 2 months in addition to management practices eliminating exposure to hay in the environment results in significant improvement in the clinical status of horses when compared to only providing low dust diet.
CHAPTER 1.

INTRODUCTION

Chronic lower airway inflammatory diseases commonly occur in horses. Recurrent airway obstruction (RAO) is more prevalent in wet, cool climates in mature to older animals, while inflammatory airway disease (IAD) can occur under any environmental condition and can affect horses of any age, but tends to be more common in the younger population.¹

No definitive cure for RAO exists, however horses affected by both diseases can be managed long term by the combination of environmental management (minimizing dust exposure by housing and dietary changes) and medical therapy (anti-inflammatory medications and bronchodilators).² ³ The recurrence rate for IAD is low, so horses suffering from this disease may achieve complete recovery with adequate long term management changes.⁴ The life-long management however, can require significant commitment (both time and financial) from owners. Ideal strict environmental control achieves maintenance of clinical remission in the majority of horses. Based on limited evidence in the human and veterinary literature, omega–3 PUFA containing feed supplements may be beneficial in improving clinical signs in horses affected by chronic lower airway inflammatory diseases.⁵–⁷
The purpose of this study was to evaluate the clinical efficacy of an omega-3 PUFA containing equine feed supplement (*Aleira™*) in improving the clinical signs in horses affected by chronic lower airway disease.
CHAPTER 2.

LITERATURE REVIEW

Chronic lower airway inflammatory diseases of horses

All equids can be affected by RAO, but females, horses older than 4 years of age and Thoroughbreds are more likely to be diagnosed based on a large scale retrospective study.\(^8\) The prevalence in North America and in Europe is estimated to be 14 % in the adult population.\(^9\), \(^10\) A familial basis for the disease has been proposed, and an association between RAO in certain horse families and particular genes has been recently identified.\(^11\) Manifestation of the disease is triggered by environmental factors, including inhaled molds and organic dust (feed, hay, straw).\(^12\) Clinical disease is characterized by excessive mucus production in the airways, neutrophil accumulation, airway hyperresponsiveness and reversible bronchospasm. Common clinical signs include increased respiratory rate and effort at rest, coughing, nasal discharge and exercise intolerance. More severely affected cases can present in severe respiratory distress characterized by markedly increased respiratory rate and expiratory effort (“heave”), nostril flaring, anxious facial expression and reluctance to move.\(^13\) While history, clinical
signs and response to treatment are suggestive of the disease; definite diagnosis is achieved by cytological analysis of the lower respiratory tract secretions and lung function testing. Horses clinically affected with RAO will have a suppurative, non-septic inflammation \(^{14}\) characterized by non-degenerative neutrophil percentage > 25% in the bronchoalveolar lavage fluid. Pulmonary function testing is a valuable tool to demonstrate reversibility of airway obstruction in horses affected with RAO and include measurements of dynamic lung compliance, pulmonary resistance, and maximal pleural pressure changes.\(^{15}\)

While RAO is a very well characterized clinical syndrome, IAD has been just recently defined as a separate clinical entity.\(^1\) Mostly younger to middle-aged horses are affected and as opposed to RAO cases, horses diagnosed with IAD have subtle to no clinical signs at rest, but exhibit decreased performance, coughing and excessive tracheal mucus accumulation. The pathogenesis of IAD is poorly defined however, noninfectious agents such as inhaled organic and inorganic particles are thought to play a central role in the development of the disease thereby causing mild to moderate allergic airway inflammation.\(^{16}\) Horses with IAD are diagnosed based on characteristic clinical signs, evidence of mucus accumulation in the trachea on airway endoscopy and cytology of bronchoalveolar lavage fluid that is characterized by mild neutrophilia (>5%), increased mast cell count (>2%) or eosinophil cell count (>1%).\(^1\) Pulmonary function testing does not provide consistently evidence for airway obstruction in horses diagnosed with IAD. Signs of airway hyperresponsiveness however, are present in horses with mastocytic or
eosinophilic inflammation, when they are exposed to nonspecific airway irritants, such as histamine aerosol.\textsuperscript{1, 16, 17}

**The role of oxidative stress in recurrent airway obstruction**

Chronic airway inflammation, mediated by the overexpression of a large number of inflammatory genes, pro-inflammatory cytokines, chemokines and adhesion factors, is the key feature of recurrent airway obstruction.\textsuperscript{18} Reactive oxygen species released by inflammatory cells have also been shown to play a role in the pathogenesis of inflammatory airway diseases.\textsuperscript{19} The end products of this process, such as lipid hydroperoxides, phospholipids, aldehydes and isoprostanes promote lung inflammation.\textsuperscript{20} Markers of oxidative stress including glutathione, glutathione disulphide and 8-isoprostane, have been shown to be increased in the BALF of RAO affected horses during an acute crisis.\textsuperscript{21} Studies have described the antioxidant status of RAO affected horses in clinical remission when compared to healthy controls,\textsuperscript{18} and the role of antioxidant supplementation on pulmonary performance and systemic anti-oxidant status.\textsuperscript{22} Based on this information, measurements of markers of oxidative stress along with levels of anti-oxidant substances (ascorbic acid and $\alpha$-tocopherol) in the peripheral blood can provide adequate assessment of the magnitude of lipid peroxidation associated with the ongoing inflammation in the lungs.
**Omega-3 polyunsaturated fatty acids in the management of respiratory inflammatory conditions in human medicine**

Increased consumption of omega-3 PUFAs, such as eicosopentaenoic acid (EPA) and docosahexanoic acid (DHA), results in greater integration of these fatty acids into the inflammatory cell phospholipids.\(^{23}\) The role of omega-3 PUFAs in inflammatory conditions is multifactorial. The incorporation of EPA and DHA occurs in a dose dependent fashion at the expense of arachidonic acid, and as a result, less substrate is available for the synthesis of eicosanoids during an inflammatory process. Another role of omega-3 PUFAs has been recently described, with the discovery of resolvins. These mediators are derived from EPA (E-series resolvins) and DHA (D-series resolvins), and have been shown to have direct anti-inflammatory properties.\(^{23}\) Additional roles of omega-3 PUFAs in inflammation include decreasing activation of nuclear factor kappa-light-chain-enhancer of activated B cells and as a result, decreased generation of inflammatory cytokines like tumor necrosis factor-\(\alpha\), interleukin-1\(\beta\) (IL-1\(\beta\)), IL-6, IL-8, and expression of adhesion molecules.

Multiple clinical studies have been undertaken to investigate the efficacy of omega-3 PUFAs in chronic pulmonary inflammatory conditions in people with variable results.\(^5,23\) A trial in asthmatic children reported significant improvement in clinical scores, sputum evaluations and pulmonary function testing after supplementation with omega-3 PUFAs for 6 weeks at a dose of 1g/day.\(^6\) A study evaluating the response to a low-dose allergen challenge in asthmatic people revealed significantly decreased bronchial inflammation in
patients receiving 630 mg omega-3 PUFAs daily for 5 weeks compared to the placebo group, while a higher dose of supplementation (780 mg/day) given only for 2 weeks didn’t improve pulmonary function in people with stable asthma. A study evaluating the effects of the daily intake of the lipid extract of the New Zealand green-lipped mussel that would provide 200 mg omega-3 PUFA daily in patients with atopic asthma, revealed a significant improvement in clinical signs (such as daytime wheezing) as well as in some lung function outcome measures (e.g. peak expiratory flow) when compared to placebo. A clinical trial in patients suffering from seasonal asthma receiving 3g omega-3 PUFAs daily for 30 days revealed a significant improvement in pulmonary function testing that lasted only for a short period after treatment was discontinued, suggestive of a need for continuous administration. Based on these current studies, it appears that the doses as well as the length of supplementation play an important role in the overall efficacy of omega-3 PUFAs in the management of chronic lower airway conditions in people.

**Omega-3 polyunsaturated fatty acids in the management of inflammatory conditions in veterinary medicine**

There are only a limited number of trials published in the veterinary literature evaluating the effects of supplementation with omega-3 PUFAs in animals. Efficacy has been shown in the management of canine atopic dermatitis, where dogs were supplemented with different preparations of omega-3 PUFAs for 10 weeks, and then clinical scores were compared to the placebo group. In this experiment, while blood levels of the
supplemented omega-3 PUFAs increased significantly, the skin concentrations of omega-3 PUFAs was not elevated and no correlation was found between clinical improvement and blood levels of the omega-3 PUFAs. A study evaluating the production of inflammatory mediators during omega-3 PUFA supplementation in healthy dogs exposed to lipopolysaccharide challenge found that the placebo group had significantly higher serum activity of IL-1, IL-6, TNFα and prostaglandin E2 when compared to the treatment group. These results suggest the ability of omega-3 PUFA supplementation to decrease the effects of inflammation. An experimental trial assessing the effects of omega-3 PUFA administration for 4 weeks on airway hyperresponsiveness and inflammation in cats with experimentally-induced asthma revealed successful integration of polyunsaturated fatty acids in erythocyte cell membranes and some beneficial effects on airway responsiveness through a leukotriene A2 dependent pathway. The most challenging aspect of omega-3 PUFA supplementation in horses is finding an effective dose that is yet reasonably priced. In a recent study, evaluating immune function of healthy yearling horses after omega-3 PUFA supplementation, the investigators found a dose of 6g/100 kg to effectively alter the composition of plasma and red blood cell phospholipids after a 35-day supplementation period. This study however did not evaluate lower doses. A randomized, cross-over trial was reported on horses diagnosed with RAO and supplemented with sunflower oil (rich in linolenic acid) or seal blubber oil (rich in omega-3 PUFAs). A 10-week supplementation period revealed successful integration of omega-3 PUFAs into leukocyte membranes and a decrease in overall leukocyte count in the BAL fluid, but no effects on clinical score or pulmonary function were detected. While this study used a high dose of daily supplementation (65g/day), the lung function
measurements were taken after administration of sedation which would confound these measurements due to the bronchodilatory properties of the commonly used sedatives in horses (e.g. α2 agonists). Therefore, sedation prior to taking the measurements could potentially result is falsely improved values. Additionally, in this study, the authors did not include clinical parameters as outcome measures, which would have been helpful to judge the efficacy of treatment on chronic airway disease, as clinical signs often improve significantly prior to seeing improvement in pulmonary function or airway cytology. It is also important to mention, that, the horses in that study were fed hay for the duration of the experiments, therefore were constantly exposed to the dusty environment.

Equine RAO and IAD present many similarities with asthma in people. Based on experiences in human asthma, feed supplements containing omega – 3 PUFAs have been proposed to improve clinical signs in horses with RAO.

The ultimate goal of our study was twofold:

1. To find the minimal effective dose of a feed supplement containing omega-3 PUFAs (Aleira™) that is able to alter the composition of phospholipid classes of plasma, in particular, increasing DHA fraction.

2. To evaluate whether supplementation with Aleira™ is able to improve clinical scores, bronchoalveolar lavage (BAL) fluid cytological profile and 8-isoprostan levels and pulmonary function in horses with chronic lower airway disease fed a low-dust diet while being maintained in the same environment.
We hypothesized that daily administration of Aleira™ to horses with chronic lower airway disease along with low-dust diet for 8 weeks will result in greater improvement in clinical score, BAL fluid variables and pulmonary function when compared to horses fed placebo and low-dust diet.

The rationale for the proposed research is that there is only limited scientific evidence that supports the beneficial effects of dietary supplementation with omega-3 PUFAs in horses affected by chronic lower airway inflammatory diseases. The data from our study would provide further information to support the usage of such supplements and specifically Aleira™.
Chronic lower airway inflammatory diseases commonly occur in horses. Recurrent airway obstruction (RAO) is more prevalent in mature to older horses stabled in wet, cool climates, while inflammatory airway disease (IAD) can occur under any environmental condition and can affect horses of any age, but tends to be more common in the younger population.\textsuperscript{1} No definitive cure for RAO exists, however horses affected by both diseases can be managed long term by a combination of environmental management (minimizing dust exposure by implementing housing and dietary changes) and medical therapy (anti-inflammatory medications and bronchodilators).\textsuperscript{2,3} The recurrence rate for IAD is low, so horses suffering from this disease may achieve complete recovery with adequate long term management changes.\textsuperscript{4} The life-long management changes required for RAO affected horses however, can demand significant commitment (both time and financial) from owners.
Increased consumption of omega-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), results in greater integration of these fatty acids into the inflammatory cell phospholipids. The role of omega-3 PUFAs in inflammatory conditions is multifactorial. The incorporation of EPA and DHA occurs in a dose dependent fashion at the expense of arachidonic acid, and as a result, less substrate is available for the synthesis of eicosanoids during an inflammatory process. Another role of omega-3 PUFAs has been recently described, with the discovery of resolvins. These mediators are derived from EPA (E-series resolvins) and DHA (D-series resolvins), and have been shown to have direct anti-inflammatory properties. Additional roles of omega-3 PUFAs in inflammation include decreasing activation of nuclear factor kappa-light-chain-enhancer of activated B cells and as a result, decreased generation of inflammatory cytokines like tumor necrosis factor-α, interleukin-1β (IL-1β), IL-6, IL-8, and expression of adhesion molecules.

Multiple clinical studies have been undertaken to investigate the efficacy of omega-3 PUFAs in chronic pulmonary inflammatory conditions in people with variable results. A trial in asthmatic children reported significant improvement in clinical scores, sputum evaluations and pulmonary function testing after supplementation with omega-3 PUFAs for 6 weeks at a dose of 1g/day. A study evaluating the response to a low-dose allergen challenge in asthmatic people revealed significantly decreased bronchial inflammation in patients receiving omega-3 PUFAs daily for 5 weeks compared to the placebo group, while a higher dose of supplementation given only for 2 weeks didn’t improve pulmonary function in people with stable asthma. A clinical trial in patients suffering from seasonal asthma receiving 3g omega-3 PUFAs daily for 30 days revealed a significant
improvement in pulmonary function testing that lasted only for a short period after treatment was discontinued, suggestive of a need for continuous administration.\textsuperscript{26}

There are only a limited number of trials published in the veterinary literature evaluating the effects of supplementation with omega-3 PUFAs in animals. Efficacy has been shown in the management of canine atopic dermatitis\textsuperscript{27} and in cats with experimentally-induced asthma.\textsuperscript{29} The most challenging aspect of omega-3 PUFA supplementation in horses is finding the effective dose that is yet reasonably priced. In a recent study, evaluating immune function of healthy yearling horses after omega-3 PUFA supplementation, the investigators found a dose of 6g/100 kg to effectively alter the composition of plasma and red blood cell phospholipids after a 35-day supplementation period.\textsuperscript{30} A randomized, cross-over trial was reported in horses diagnosed with RAO and supplemented with sunflower oil (rich in omega-6 PUFAs) or seal blubber oil (rich in omega-3 PUFAs).\textsuperscript{31} A 10-week supplementation period revealed successful integration of omega-3 PUFAs into leukocyte membranes and a decrease in overall leukocyte count in the BAL fluid, but no effects on clinical score or pulmonary function. While this study used a high dose of daily supplementation (65g/day), administration of sedation confounded the pulmonary function testing results questionable.\textsuperscript{31}

The purpose of the study was to evaluate the clinical efficacy of an omega-3 PUFA-rich equine feed supplement in horses affected by chronic lower airway disease. We hypothesized that daily administration of the omega-3 PUFA supplement to horses with chronic lower airway disease along with low-dust diet for 8 weeks will result in greater
improvement in clinical score, BAL fluid variables and pulmonary function when compared to horses fed placebo and low-dust diet.

MATERIALS AND METHODS

Experimental design, animals

Pilot study: four healthy horses and four previously diagnosed RAO affected horses were selected from the Purdue University Herd to participate in the dose determination study. They were divided into 2 groups: Group 1, encompassing 2 healthy and 2 RAO affected horses, was started on the omega-3 PUFA supplementation at the lowest dose recommended by the manufacturer (1X= 30 g/day); Group 2, encompassing 2 healthy and 2 RAO affected horses, was started on the omega-3 PUFA supplementation at a double dose (2X= 60 g/day). Horses underwent a thorough physical examination before enrollment into the study and venous blood samples were collected to determine serum phospholipid profiles.

Horses were monitored daily during the trial for general attitude and appetite. Physical examination including clinical scoring was performed weekly. Blood was collected every 2 weeks to determine plasma phospholipid profiles. After the first 2 weeks, the dosages were doubled (the group receiving the supplement at a 1x dose was continued at a 2x dose, while the groups receiving the supplement at a 2x dose was continued at a 4x dose). Horses then were maintained on the same dose and blood samples continued to be
collected every 2 weeks to determine plasma phospholipid profiles, until < 10% change in omega-3 PUFA ratio was identified compared to previous measurements, suggestive of a plateau effect. Once plateau was reached, supplement administration was discontinued.

Efficacy study: Sample size calculation based on previously published clinical data from client applied visual analog scoring (VAS)\textsuperscript{33} showed that $n \geq 32$ horses would be needed to allow detection of 30% improvement in VAS (breathing score and cough) post-treatment with an 80% power and a significance level $\alpha = 0.05$. The study was performed over a 9-month period as a randomized, placebo-controlled, double-blinded clinical trial. Client-owned horses were recruited by advertisements and mails sent out to referring veterinarians and brought to the Purdue University Veterinary Teaching Hospital (PUVTH) for testing. Horses were enrolled if they had history of chronic respiratory disease of at least 3-month duration and exhibited clinical signs of lower airway inflammation at the time of recruitment with at least one of the following: increased breathing rate or effort at rest, intermittent cough, or poor performance. After obtaining client consent, horses underwent a thorough physical examination including clinical scoring, blood collection, pulmonary function testing and BAL fluid collection on the day of enrollment (day 0). Only horses with a normal leukogram and no clinical evidence of infectious respiratory disease were included in the study. Horses then returned home after they were randomly assigned to receive one of three treatments (omega-3 PUFA supplement 1x or 2x, or placebo) as a daily feed supplement for 8 weeks, while their attitude and appetite were monitored daily by the owners. They were maintained in the same environment as before enrollment into the study but their diet was
switched to complete pelleted feed\(^a\) and no exposure to hay was authorized during the study period. Coupons for free bags of feed were given to owners to cover needs during the trial and compliance to these rules was assessed during history taking at recheck visit. All medications were withheld during the study period. Owners were asked to assign a visual analog score (VAS, see Appendix A and B) for performance, breathing difficulty and cough upon enrollment and then once a week (day 7, 14, 21, 28, 35, 42, 49 and 56).\(^{34}\)

At the end of the supplementation period (day 56), horses returned to PUVTH to undergo the same procedures as on Day 0.

Horses were diagnosed with IAD based on BAL fluid cytology showing either >1% eosinophil count, >2% mastocyte count or >10% neutrophil count along with characteristic clinical signs (cough, exercise intolerance), and a maximum change in transpulmonary pressure (\(\Delta P_{\text{Lmax}}\)) less than 15 cmH\(_2\)O, while horses with chronic respiratory disease and \(\Delta P_{\text{Lmax}} > 15\) cmH\(_2\)O were diagnosed with RAO.

The Purdue University Animal Care and Use Committee approved the study.

**Feed supplement administration and housing**

*Pilot study:* horses were maintained in outside dirt paddocks and were brought in a stall to receive the feed supplement as a top dressing on complete pelleted feed\(^a\) fed at 2% of bodyweight daily.

*Efficacy study:* horses were randomly selected by an online randomization generator\(^b\) to receive the omega-3 PUFA feed supplement (1x or 2x dose) or placebo as a
top dressing on pelleted feed once daily for 8 weeks. During the study period horses were fed a diet of complete pelleted feed\(^a\) based on individual energy requirements (1.5 – 2% of bodyweight) with no access to hay.

**Clinical evaluation**

Physical examination was performed weekly during the pilot study, while it was performed twice on client owned horses (day 0 & 56). Physical examination included evaluation of hydration and perfusion parameters, cardiac and thoracic auscultation, rectal temperature, and body weight.

Two previously adopted clinical scoring systems were used to assess respiratory compromise in the efficacy study at the time of initial assessment and at the end of the supplementation period based on 1) respiratory rate, respiratory effort, nasal discharge, and presence or absence of cough and abnormal lung sounds (range 0-21); 2) abdominal effort and nostril flaring (range 2-8). (Appendix C)\(^{35, 36}\) Additionally, owners were instructed on how to quantify performance, breathing difficulty and cough using a visual analog scale (VAS, score 0-100) and were asked to record it weekly from day 0 to 56 (Appendix A, B).\(^{34}\) Qualification for enrollment in the study included a VAS score of 70 or less in at least one of the 3 categories with a score of 100 indicating absence of clinical signs.
Venipuncture was performed on the left or the right external jugular vein with a 20G x1.5-inch needle connected to an evacuated tube containing EDTA. Complete blood count analysis was performed on a commercially available automated hematology analyzer. Plasma samples were centrifuged 10 minutes at 3400g and stored at -80 °C until further processing.

Fatty acid analysis of plasma was carried out on freshly thawed sample after extraction of lipids by the Folch method\textsuperscript{37}, isolation of phospholipids by thin layer chromatography, methylation utilizing boron trifluoride and gas chromatography as described previously.\textsuperscript{38}

**Pulmonary function testing (PFT)**

Horses were restrained in stocks without sedation. An esophageal balloon catheter (inside diameter, 4.8 mm; outside diameter, 6.4 mm; length, 240 cm) was advanced through the nose to mid-thorax as described before.\textsuperscript{15} The exact position of the catheter was recorded for each horse at baseline testing and used in the subsequent measurements. A second catheter of same size and diameter was used to measure pressure within the mask and both catheters were connected to a differential pressure transducer.\textsuperscript{c} Maximum change in transpulmonary pressure ($\Delta P_{L_{\text{max}}}$) was defined as the difference between pleural and mask pressures during peak inspiratory and expiratory effort. A mask was placed over the nose of each horse with a pneumotachometer\textsuperscript{d} coupled to a differential pressure transducer\textsuperscript{e} that measured a signal proportional to airflow. Signals produced by the pneumotachometer and the pressure transducers were recorded by computer software.
Resistance ($R_L$) was measured using the isovolume 50% method. Dynamic compliance ($C_{dyn}$) was computed by dividing tidal volume by the difference in $\Delta P_{L_{max}}$ between points of zero flow.

After completion of standard lung function mechanics, horses were sedated with a combination of detomidine (0.02 mg/kg) and butorphanol (0.02 mg/kg) in preparation for the BAL.

**Bronchoalveolar lavage (BAL) fluid sampling and analysis**

Bronchoalveolar lavage was performed with a BAL tube after lung function measurements were obtained.\textsuperscript{15} Tubing and containers in contact with BAL fluid were sterile. The caudo-dorsal area of the left or right diaphragmatic lobes was sampled at random. Five hundred milliliters of sterile 0.9% NaCl was infused and retrieved from the airways in 3 aliquots. The BAL fluid was immediately placed on ice and processed within 20 minutes of collection. Cytological specimens were prepared by centrifugation and processed with Wright’s stain. For 8-isoprostane concentrations, aliquots of 2ml BAL treated with 0.005% butylated hydroxytoluene were stored at -80C until analysis via an enzyme immune assay, using an enzyme immunoassay kit\textsuperscript{18} validated in the horse. The BAL tube was cleaned and sterilized after each procedure.
**Data analysis**

Data distribution was assessed using the Shapiro-Wilk test for normality. The data in the pilot study were not normally distributed. The effect of dose (1x vs. 2x) and disease status (healthy vs. RAO) was tested by analysis of covariance (ANCOVA) using post-treatment variable as outcome variable and pre-treatment variable as covariate. If significant effect was detected then, post-hoc analysis was conducted using Friedman analysis of variance (ANOVA) when comparing repeated measurements between week 0 and 8 or Mann-Whitney U test when comparing between groups at a given time point.

Data from the efficacy study were not normally distributed. The effect of dose (placebo vs. 1x vs. 2x) and disease status (IAD vs. RAO) were tested using ANCOVA. Data were compared between baseline and 2 months following treatment with Wilcoxon matched pairs tests. Data were compared between treatment groups at each time point using Kruskal-Wallis ANOVA (3 treatment groups: Placebo, 1x, 2x) or Mann-Whitney U test (2 treatment groups: Placebo, treatment [1x or 2x]). Owner-assigned scores (VAS) between week 0 and 8 were compared using Friedman ANOVA. Post-hoc paired comparisons of VAS scores between week 0 and subsequent weeks were performed using Wilcoxon matched pairs tests with Bonferroni adjusted p-value (0.05/7=0.0071; P<0.0071).

All data were expressed as median [25% - 75% quartiles]. P<0.05 was considered significant except for comparison of weekly VAS scores were P<0.0071 was considered significant.
RESULTS

Pilot study

All horses (n=8) enrolled in the pilot study remained healthy throughout the experiments. There were 5 geldings and 3 mares in the study, aged between 11-26 years. Regarding the breed distribution, there were 3 Quarter Horses, 2 Paints and one of each: Appaloosa, Thoroughbred, Tennessee Walking Horse. All RAO affected horses involved in this trial remained in clinical remission, there were no differences between physical exam findings and plasma lipid proportions between the healthy and RAO affected horses, therefore the data from both groups were pooled. The only significant difference between the groups receiving the different doses (1x vs. 2x) was for oleic acid (18:1n9c) where relative plasma levels decreased in horses receiving 2x dose during 4 weeks (P=0.018) but did not change in those fed 1x dose.

Subsequently, data from horses receiving 1x or 2x Aleira were pooled. Horses receiving the 2x dose of Aleira were only supplemented for 4 weeks, as their PUFA profile did not differ from the 1x group. The relative amount of DHA (expressed as % of total fatty acids) increased significantly between week 0 and 4 of supplementation and then reached a plateau between week 4 and 8 (P=0.012).

Efficacy study

There were a total of 35 horses evaluated for the dose efficacy study. Out of these horses, 34 qualified, as there was one horse that was diagnosed with upper respiratory tract
obstruction on the initial evaluation, and 32 horses completed the trial. One of the horses that dropped out died of colic approximately 1 month after enrollment in the study. No necropsy was performed due to lack of consent from the owner, but the horse was reported to be well up until the onset of colic, and according to the VAS scoring sheet, showed clinical improvement in his respiratory signs. Therefore we believe that the colic was unrelated to the supplement administration. Another horse was excluded from the final data analysis due to lack of compliance from the owners, as they disclosed that they started feeding hay again during the trial.

Regarding the sex distribution, there were 18 geldings, 11 mares and 3 stallions. The age of the horses ranged between 6-22 years. Regarding breed distribution, there were 13 Quarter Horses, 6 Walking Horses, 3 Arabians, 2 Appaloosas and one of each: Thoroughbred, Mustang, Saddlebred, Warmblood and Paint. Three horses were of unknown breed.

Eleven horses received placebo feed supplement, ten were fed the supplement at the recommended label dose (1x) and eleven horses received twice the labeled dose (2x). All the horses included in the data analysis were maintained on a complete pelleted feed for the entire duration of the study, with no exposure to hay. None of the owners reported any adverse effects related to the supplementation, all horses accepted the supplement or placebo well, and consumed it readily mixed with the pelleted feed.

Fourteen horses were diagnosed with RAO and 18 with IAD based on clinical evaluation, lung function testing and cytological analysis of the bronchoalveolar fluid. On baseline evaluation, there was a statistically significant difference between IAD and RAO cases in
regards to pulmonary function test results, such as $\Delta P_{\text{max}}$ (8.9 ± 3.0 and 26.6 ± 15.8 cmH$_2$O in IAD and RAO horses, respectively; $P<0.0001$), $C_{\text{dyn}}$ (1.9 ± 0.8 and 1.2 ± 0.7 L/cmH$_2$O in IAD and RAO horses, respectively; $P=0.02$) and $R_L$ (0.7 ± 0.3 and 1.9 ± 1.2 cmH$_2$O/L/s in IAD and RAO cmH$_2$O horses, respectively; $P<0.001$). Additionally, the 2 groups differed in regards to the neutrophil granulocyte percentage in the BAL fluid ($P=0.01$). Other clinical parameters, such as the veterinarian assigned clinical scores, owner assigned VAS and physical exam findings; the fatty acid profiles and 8-isoprostane levels in the BAL fluid were not different between the 2 groups at baseline evaluation. No difference in treatment effect was detected between horses with RAO or IAD horses therefore; data analysis was subsequently performed on data pooled among all horses with chronic respiratory disease.

At baseline, none of the variables were different between placebo and treatment groups. Data analysis with the 3 different groups (placebo, 1x, 2x) revealed only one significant treatment effect (VAS cough). Post-hoc analysis revealed that VAS cough improved significantly (i.e. increased) in all 3 treatment groups (placebo, 1x, 2x) however, horses treated with 1x dose of omega-3 PUFA supplement exhibited a significantly higher VAS cough score 2 months later than horses receiving placebo ($P=0.043$). (Figure 1.) As there was no additional effect associated with the different doses of supplementation noted, data from horses treated with 1x and 2x dose of omega-3 PUFA supplement were pooled and compared to data from horses treated with placebo.
Clinical signs - The effect of treatment with the supplement on clinical signs was statistically significant as compared to placebo. Post-hoc analyses showed that clinician assigned clinical scores (both long and short scores) in horses treated with the omega-3 PUFA supplement (1x or 2x) exhibited a significant improvement (P<0.001) however; scores at the end of supplementation were not different between the placebo and treatment groups (Figure 2). When evaluating the owner-assigned clinical scores (VAS cough, respiratory effort, performance), they all improved significantly in the placebo and treatment horses over time, however VAS cough and VAS respiratory effort scores 2 months after treatment showed a significantly greater improvement in horses given the omega-3 PUFA supplement compared to placebo (P=0.031) (Figure 3). Clinical data is summarized in Table 1.

According to the VAS scoring sheets filled out by the owners on a weekly basis, the effect of supplementation and low dust diet on clinical signs were noticeable during the first 2 weeks of therapy and reached maximum benefit between weeks 2-5 for coughing, weeks 5-6 for respiratory effort and weeks 3-5 for poor performance (Figure 4).

BAL fluid analysis – Omega-3 PUFA supplementation for 2 months resulted in a significant decrease in the relative proportion of inflammatory cells (neutrophils) in the lung mucus, and this effect was not observed in horses fed the placebo (Figure 5; Table 1). Isoprostane concentrations in BAL fluid were not significantly affected by treatment. (Table 1.)

Lung function testing - Two months of supplementation improved lung function (decreased $\Delta P_{L,max}$ and $R_L$) in both placebo and omega-3 PUFA treated horses however,
the effect only reached statistical significance in horses fed the omega-3 PUFA supplement (P<0.01) (Figure 6).

*Phospholipid analysis of plasma* - The only fatty acid that was significantly affected by omega-3 PUFA treatment was DHA whereby 2 month of supplementation resulted in a 59% increase (P<0.001; Figure 7). No significant increase was detected in the placebo treated horses.

**DISCUSSION**

This study is the first report evaluating the effects of an omega-3 PUFA containing feed supplement administered to horses affected with chronic lower airway inflammatory disease and concurrently fed a low dust diet. The pilot study confirmed that the dose recommended by the manufacturer (30 g/day) is able to alter the phospholipid classes of plasma, and results in a rise of DHA levels. Results from the double-blind, placebo-controlled trial showed that administration of the omega-3 PUFA supplement provided additional clinical benefits when compared to the control group maintained in a low dust environment and fed placebo.

In this study we elected to use both horses diagnosed with RAO and IAD. We were interested to see, if there would be a difference in response to omega-3 PUFA administration with the different chronic lower airway inflammatory diseases. At baseline evaluation, the difference in disease status was clearly seen in pulmonary function test results and cytological analysis of the BAL fluid, while overall clinical assessment was
not different between the RAO and IAD groups. This is not unexpected, as horses with IAD do not have evidence of airway obstruction based on standard lung mechanics\textsuperscript{15} and often have mastocytic and eosinophilic inflammation present in the BAL fluid\textsuperscript{1}, compared to RAO horses, that have increased $\Delta P_{L_{\text{max}}}$ and $R_L$ and severe neutrophilic inflammation in BAL fluid.\textsuperscript{10} As we were mostly interested in clinical response to the supplementation and there were no differences in treatment effect based on disease category, we elected to pool data from RAO and IAD horses and perform data analysis regardless of disease status.

A previous study evaluated omega-3 PUFA supplementation in horses with RAO,\textsuperscript{31} but failed to detect a significant treatment effect in pulmonary function. The study used a high dose of supplementation (65 g/day) especially when compared to our study, where a lower dose of the omega-3 PUFA supplement did result in significant treatment effect. In that experiment, however horses received sedation (detomidine and butorphanol) before performance of lung function testing that may have contributed to the negative overall results, as the sedatives used can result in bronchodilation.\textsuperscript{32, 40} Additionally, horses were fed hay throughout the study period, so they were continuously exposed to the dusty environment which would likely diminish any potential benefit provided by PUFA supplementation. In the present study, improvement was noted in horses receiving omega-3 PUFA supplementation regarding clinical parameters, including the veterinarian assigned clinical score that evaluated the degree of respiratory compromise, the owner assigned VAS that evaluated the coughing and the respiratory effort of the horse, and the number of inflammatory cells in the BAL fluid as well as in the lung function. Limiting
dust exposure in the environment by removing hay from the diet results in clinical improvement in horses with lower airway inflammatory diseases, so some degree of improvement was expected in all treatment groups just by switching the horses’ diet from hay to pelleted feed. We thought this design would allow a more accurate evaluation of the effect of omega-3 PUFA supplementation, as horses in severe crisis would have been unlikely to show clinical improvement just with the feed supplement administration. This experimental design would also standardize the influence of dietary difference between horses and represent the typical management change that practitioners would recommend in the field.

Owner assessment of clinical signs in horses with chronic airway disease has been previously reported by Gerber et al to be successful in judging efficacy of a certain treatment. We used data from that study for sample size calculation in order to detect a 30% improvement in VAS scores that was deemed clinically relevant. When comparing the efficacy of the omega-3 PUFA supplement and environmental modification to the dexamethasone treated group in Gerber’s study, interestingly the magnitude of improvement in the VAS cough and respiratory effort scores is comparable between the two studies. This result is surprising, and suggests that the low dust diet along with the omega-3 PUFA supplement administered to horses with chronic airway disease for 8 weeks have similar benefits as a shorter course (3 weeks) of dexamethasone administration (0.1 mg/kg, q24h). This effect is most likely due to the combination of allowing the respiratory tract to clear the allergens by limiting exposure as seen in the placebo group and allowing adequate integration of omega-3 PUFAs into the
inflammatory cell membranes and altering the inflammation over an extended period of time.

Inflammation in an acute RAO crisis has been associated with increased levels of markers of oxidative stress in the airways. Interestingly, measurements of 8-isoprostane from the BAL fluid did not reveal any changes when compared to baseline levels in our study. One possible explanation is that oxidative stress in the airways was not directly affected by the treatment, or it is possible that 8-isoprostane levels did not accurately reflect the redox state of the horses in the study, and measurements of other markers of oxidative stress such as reduced or oxidized glutathione would have been more accurate for such evaluation. It is also possible, that the low dose supplement was able to modify some aspects of the inflammatory response responsible for clinical improvement, without affecting oxidative stress. In a follow up project, it would be interesting to evaluate cytokine levels in the BAL fluid before and after supplementation, to see if a cause and effect relationship can be identified.

Measurement of fatty acid classes in plasma was also performed, and revealed a statistically significant increase in plasma DHA levels in the treatment group when compared to baseline values, which was not observed in the placebo group. These results of the fatty acid analysis allow us to believe that the increase in DHA levels in blood could have been responsible for the clinical improvement in the horses receiving the supplement, when compared to the placebo group. The DHA dose used in this study was based on the recommendations of the manufacturer of the product. According to label, the daily recommended dose of 30 grams of the supplement that is recommended daily,
contains 1.5 grams of DHA per serving. Additionally to the dose recommended by the manufacturer, we evaluated administration of a double dose to determine whether a higher dose would result in additional improvement of clinical signs. It was concluded that a single dose of the supplement (1 scoop = 30 grams) appeared to be as beneficial as the double dose (2 scoops = 60 grams). No additional benefits in clinical signs, lung function or cytological analysis of the BAL fluid were noted with the increased dose. This finding is interesting, as it has been shown previously that, incorporation of omega-3 PUFAs into the inflammatory cell membrane occur in a dose dependent fashion\textsuperscript{41}, therefore a greater improvement would be expected with the higher dose. The dose of 1.5 grams DHA/day was able to alter the phospholipid classes of plasma, and resulted in a significant rise in DHA % in plasma in both pilot and clinical trial, despite that the DHA dose used in this study was much lower than reported previously in horses.\textsuperscript{30, 42, 43} The experiments in the literature however did not include lower doses to evaluate integration of omega-3 PUFA in blood. According to our findings, feeding the 2x dose did not proportionally increase plasma levels of DHA. It is possible, that there is a limit of how much omega-3 PUFA can be absorbed and integrated in the horse and higher doses of supplementation do not carry additional health benefits. Another possible explanation is that the source of the omega-3 PUFA makes a difference. The supplement used in our study was from an algae source, which has not been evaluated in horses before.

It is important to note that there were other ingredients in the supplement, such as Vitamin C, methylsulfonylmethane and mushroom complex which may have contributed to decreasing inflammation. MSM has been shown to improve clinical signs of
inflammation in people with hay fever,\textsuperscript{44} while Vitamin C has been proposed to decrease exercise induced bronchoconstriction in people.\textsuperscript{45} However, vitamin C was also included in the placebo product. According to the manufacturer, the proprietary mushroom complex is supposed to provide additional antioxidant properties to the supplement. Since oxidative stress measurement using 8-isoprostane didn’t reveal any difference between placebo and treatment group, it is unlikely that clinical benefits observed in the treatment group were due to the mushroom complex rather than the DHA supplementation. Measurements of additional oxidative stress and inflammatory markers in blood may have allowed us to draw more definitive conclusions on the effect on these other compounds as compared to omega-3 PUFA supplementation.

When evaluating a feed supplement to incorporate in the daily practice for life-long management, palatability and ease of administration are key factors. Horses in this study readily accepted the supplement, and owners reported it to be easy to administer, making it feasible for long term, convenient administration. Results from this study are comparable to other studies, where inhaled steroids and low dust diet were used to achieve and maintain clinical remission in horses.\textsuperscript{2} When the ease of administration is taken into consideration, horse owners are more likely to be compliant with adding a supplement to the feed, than administrating medications to the horse on a daily basis (oral or inhaled), not to mention the potential side effects associated with long term steroid administration.\textsuperscript{46}

It has been shown previously, that horses suffering from chronic lower airway inflammatory disease (both RAO and IAD) greatly benefit from reducing dust in the
environment, however some horses even on strict environmental control require rescue medications when experiencing a flare up. Based on the present study, a natural omega-3 PUFA containing feed supplement, could be an additional option to help manage these horses long term, and maintain them in a more stable remission, when compared to the low dust environment by itself. Future studies should determine the optimal duration of supplementation that result in resolution of pulmonary inflammation and whether the supplement may prevent exacerbation of respiratory disease in horses exposed to dusty environment. In order to find a direct relationship between the omega-3 PUFA administration and the clinical improvement, a future study may focus on measurements of markers of inflammation, and cytokines in the BAL fluid. Additionally, it would be important to evaluate, whether administration of a lower dose would be similarly effective, decreasing the cost associated with the continuous administration.
FIGURES AND TABLES

Table 1: Summarized data from the efficacy study. * Statistically significant difference between placebo and treatment. † Statistically significant difference between before and after supplementation.

<table>
<thead>
<tr>
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<th>Treatment</th>
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<td>8.8±4.0</td>
<td>3.4±2.1†</td>
</tr>
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<td>3.8±1.5</td>
<td>4.6±1.7</td>
<td>2.9±1.1†</td>
</tr>
<tr>
<td>VAS cough</td>
<td>39.2±22.3</td>
<td>68.8±30.6†</td>
<td>46.7±29.0</td>
<td>89.4±19.5†*</td>
</tr>
<tr>
<td>VAS respiratory effort</td>
<td>43.3±21.9</td>
<td>68.4±25.5†</td>
<td>52.8±32.0</td>
<td>85.8±23.2†*</td>
</tr>
<tr>
<td>VAS performance</td>
<td>40.0±29.2</td>
<td>65.4±30.7†</td>
<td>56.0±36.8</td>
<td>80.0±30.9†</td>
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<td>ΔPmax (cmH₂O)</td>
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<td>19.5±15.9</td>
<td>10.1±6.2†</td>
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<td>BAL Neutrophil%</td>
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<td>15.4±19.8†</td>
</tr>
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<td>DHA % in plasma</td>
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<td>0.01±0.11</td>
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<td>85.8±23.2</td>
</tr>
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<td>10.1±6.2</td>
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<td>R_l (cmH₂O/L/s)</td>
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<td>0.63±0.52</td>
<td>1.5±1.2</td>
<td>0.75±0.48</td>
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<tr>
<td>Cdyn (L/cmH₂O)</td>
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<td>2.5±1.5</td>
<td>1.3±0.7</td>
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<td>15.4±19.8</td>
</tr>
<tr>
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<td>0.1±0.09</td>
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<td>17.2±11.8</td>
<td>16.3±9.2</td>
<td>18.4±11.9</td>
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</table>
**Figure 1**: VAS cough before and 2 month after daily treatment with placebo or 1x or 2x dose of the omega-3 supplement. * Significantly different from baseline (week 0; P<0.05).

† Significantly different from placebo at week 8 (P=0.043).
Figure 2: Clinician-assigned long score (range: 0-21) in horses with chronic respiratory disease treated for 2 months with daily placebo or the omega-3 supplement. * Significantly different from baseline (week 0; P<0.05).
**Figure 3**: Effect of feed supplementation on VAS cough score of horses with chronic respiratory disease treated for 2 months. * Significantly different from week 0 (P<0.05). † Significantly different from placebo (P=0.031).
Figure 4: Weekly change in VAS cough (A) and respiratory effort (B) scores in horses fed a daily placebo or the omega-3 supplement. * Significantly different from week 0 (P<0.0071).
Figure 5: Effect of feed supplementation on the proportion of inflammatory cells (neutrophils) in the bronchoalveolar lavage (BAL) fluid of horses with chronic respiratory disease treated for 2 months. * Significantly different from week 0 (P=0.036).
Figure 6: Effect of feed supplementation on maximum transpulmonary pressure change ($\Delta P_{L\text{max}}$) in horses with chronic respiratory disease treated for 2 months. * Significantly different from week 0 (P=0.0065).
Figure 7: DHA level expressed as % of total fatty acids in plasma sample of horses with chronic respiratory disease treated for 2 months. * Significantly different from week 0 (P<0.001).
Footnotes:

\(^a\) Equine Senior, Purina Mills

\(^b\) www.random.org

\(^c\) DP/45-30, Validyne Engineering Corp, Northridge, CA

\(^d\) No. 4 Fleisch, EMKA Technologies, Paris, France

\(^e\) DP/45-14, Validyne Engineering Corp, Northridge, CA

\(^f\) Cayman Chemical Co
• The omega-3 PUFA feed supplement is safe to be used in horses, all horses accepted the supplement, and consumed it readily mixed with the pelleted feed. No adverse effects were seen in any of the research horses or reported by the clients.

• Our pilot study confirmed that the dose recommended by the manufacturer (30 grams of the supplement daily, containing 1.5 grams of DHA) is able to alter the phospholipid classes of plasma, and results in a rise of DHA levels. Results from the double-blind, placebo-controlled trial showed that administration of the omega-3 PUFA supplement provided additional benefits when compared to the control group maintained in a low dust environment and fed placebo.

• In our study, statistically significant improvement was noted in multiple clinical parameters, including the veterinarian assigned clinical score that evaluated the degree of respiratory compromise; the owner assigned VAS that evaluated the coughing and the respiratory effort of the horse, the number of inflammatory cells in the BAL fluid as well as the lung function.
• The magnitude of improvement in the VAS cough and respiratory effort scores is comparable to the effect of dexamethasone therapy in RAO horses in previous studies. This suggests that the low dust diet along with the omega-3 PUFA supplement administered to horses with chronic airway disease for 8 weeks have similar benefits as a shorter course (3 weeks) of dexamethasone administration.

• Measurements of 8-isoprostane from the BAL fluid (which has been identified as a marker of oxidative stress, present in high concentration in the BAL fluid of horses with RAO) did not reveal any changes when compared to baseline levels in our study.

• Measurement of fatty acid classes in plasma revealed a statistically significant increase in plasma DHA levels in the treatment group when compared to baseline values, which was not observed in the placebo group. These results of the fatty acid analysis suggest that the increase in DHA levels in blood could have been responsible for the clinical improvement in the horses receiving the supplement, when compared to the placebo group.

• No additional benefits in clinical signs, lung function or cytological analysis of the BAL fluid were noted with doubling the dose of daily supplementation.

• Based on the present study, a natural omega-3 PUFA containing feed supplement, could be an additional alternative to manage horses with RAO and IAD long term, and maintain them in a more stable remission, when compared to the low dust environment by itself.

• Future studies should determine the optimal duration of Aleira supplementation that result in resolution of pulmonary inflammation and whether the supplement
may prevent exacerbation of respiratory disease in horses exposed to dusty environment.

- It would be also important to evaluate, whether administration of a lower dose would be similarly effective, decreasing the cost associated with the continuous administration.
BIBLIOGRAPHY


APPENDICES
Appendix A: Page 1 of the VAS scoring sheet sent home with the owners.

Please score your horse’s health status based on these 3 parameters 1x/week during the study. Use the guidelines detailed on the other side of this paper to assign a score.

Please bring this form with you to the recheck appointment.

Assign a score between 0 – 100.

In all categories, a higher score is associated with a better health status.

<table>
<thead>
<tr>
<th></th>
<th>Date</th>
<th>Coughing</th>
<th>Respiratory effort</th>
<th>Exercise intolerance</th>
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<tr>
<td>Week 8</td>
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</tbody>
</table>
Appendix B: Page 2 of the VAS scoring sheet sent home with owners.

**COUGHING:**

Permanent, severe: Score 0
Occasional, after work or feeding: Score 50
None ever: Score 100

**RESPIRATORY EFFORT:**

Poor, in severe distress all the time: Score 0
Minimally acceptable: Score 50
Excellent, no distress ever: Score 100

**PERFORMANCE:**

Poor: Score 0
Minimally acceptable: Score 50
Excellent: Score 100
Appendix C1: Form for the clinician assigned clinical score – Long score

Clinical scoring system (adapted from Tesarowski, 1996)

<table>
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</tr>
<tr>
<td>16-20</td>
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</tr>
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<td>21-25</td>
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<tr>
<td>26-29</td>
<td>3</td>
</tr>
<tr>
<td>&gt;30</td>
<td>4</td>
</tr>
<tr>
<td><strong>Nasal discharge</strong></td>
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<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Serous</td>
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</tr>
<tr>
<td>Mucopurulent</td>
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<tr>
<td><strong>Abdominal lift</strong></td>
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<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Mild (perceptible heave line)</td>
<td>1</td>
</tr>
<tr>
<td>Pronounced (abdomen, thorax, and anal movement)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Nasal flaring</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td><strong>Tracheal sounds</strong></td>
<td></td>
</tr>
<tr>
<td>Normal (tubular sound)</td>
<td>0</td>
</tr>
<tr>
<td>Increase in intensity</td>
<td>1</td>
</tr>
<tr>
<td>Mucus movement</td>
<td>3</td>
</tr>
<tr>
<td><strong>Crackles</strong></td>
<td></td>
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<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>2</td>
</tr>
<tr>
<td><strong>Wheezes</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>2</td>
</tr>
<tr>
<td><strong>Cough</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Intermittent</td>
<td>1</td>
</tr>
<tr>
<td>Paroxysmal</td>
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</table>
Appendix C2: Form for the clinician assigned clinical score - Short score.

Clinical scoring system (adapted from Rush, 1998)

*Circle the score that applies to the horse*

a) Nostril flaring

- Normal, no signs: 1
- Slight, occasional flaring of nostrils: 2
- Moderate nostril flaring: 3
- Severe continuous flaring during each respiration: 4

b) Abdominal effort of exhalation

- Normal, no signs: 1
- Slight abdominal component: 2
- Moderate abdominal component: 3
- Severe, marked abdominal component: 4

Total score (a + b)