Randomized, controlled study of inhaled fluticasone propionate, oral administration of prednisone, and environmental management of horses with recurrent airway obstruction

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Objective—To determine whether administration of glucocorticoids provides additional benefits to environmental management of horses with recurrent airway obstruction (RAO).

Animals—28 horses with RAO.

Procedure—Horses were classified as having mild, moderate, or severe RAO. Within each category, horses were randomly assigned to receive inhaled fluticasone propionate, inhaled control substance, or oral administration of prednisone. During the 4-week study, horses were maintained outdoors and fed a pelleted feed. Clinical scores, pulmonary function, results of cytologic examination of bronchoalveolar lavage fluid (BALF), and adrenal gland function were determined before and 2 and 4 weeks after initiation of treatment.

Results—Clinical score and pulmonary function of all RAO-affected horses improved during the treatment period. After 4 weeks, clinical scores and pulmonary function of horses treated with a glucocorticoid were not different from those for the control treatment. In horses with severe RAO, treatment with fluticasone for 2 weeks resulted in significantly greater improvement in pulmonary function, compared with pulmonary function after treatment with prednisone or the control substance. Treatment with a glucocorticoid for 4 weeks and a low-dust environment did not have any effect on cellular content of BALF. Treatment with prednisone for 2 weeks resulted in a significant decrease in serum cortisol concentration, compared with concentrations after administration of fluticasone or the control substance.

Conclusions and Clinical Relevance—Environmental management is the most important factor in the treatment of horses with RAO. Early treatment with inhaled fluticasone can help accelerate recovery of horses with severe RAO. (*Am J Vet Res* 2005;66:1665–1674)

Recurrent airway obstruction (**RAO**; ie, heaves) is a syndrome characterized by chronic intermittent coughing, serous to mucopurulent nasal discharge, increased respiratory efforts, and exercise intolerance.[1] Clinical signs result from airway obstruction attributable to bronchospasm, mucus plugging of airways, and inflammatory cell infiltration that is characterized by severe neutrophilia of the **bronchoalveolar lavage fluid** (**BALF**).[2-6] This is accompanied by abnormalities in pulmonary function, such as increases in **maximum change in transpulmonary pressure** (ΔP_{Lmax}) and **pulmonary resistance** (R_L) and decreases in **dynamic compliance** (C_{dyn}).[5,7]

Horses and ponies exposed to dust or housed in poorly ventilated barns are more likely to have RAO.[8] Although the exact cause of RAO is unknown, clinical signs of RAO are exacerbated when susceptible horses are experimentally exposed to molds contained in hay for a period of a few days. In those susceptible horses experimentally exposed to molds, clinical signs and pulmonary function usually return to baseline values within 1 week after the horses are moved to pasture.[5,7] Similarly, results for cytologic examination of BALF return to reference ranges; however, some horses may have persistent neutrophilia in the airways.[9] Therefore, the first step in treating horses with RAO is to decrease their exposure to organic dust. The optimal environment is grass pasture; however, some horses may still have abnormal pulmonary function and airway inflammation after being maintained on pasture for weeks to months.[7,9,10] Another option is to treat horses with RAO by administering glucocorticoids. Treatment of horses with RAO with glucocorticoids is effective for controlling clinical signs and decreasing neutrophilia in airways.[11,12] However, improvements in respiratory efforts and airway inflammation are incomplete and short-lived when RAO-affected horses are maintained in a

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dusty environment.[13,14] The possibility that maintaining horses with RAO in a low-dust environment in combination with glucocorticoid administration may accelerate recovery has received little attention.[15]

Oral administration of prednisone is often used in clinical practice and may help reduce airway inflammation.[15] However, its effect on pulmonary function is probably marginal, presumably because of poor bioavailability.[12,15,16] Treatment of horses with RAO by inhalation of aerosolized glucocorticoids is often advocated because it decreases the dose required while achieving an optimal drug concentration in the airways. Inhaled beclomethasone dipropionate and fluticasone propionate have been effective in controlled studies[13,14,17] for the treatment of horses with RAO. To our knowledge, whether inhaled fluticasone provides additional benefit to environmental management for the treatment of RAO-affected horses has not been evaluated.

Systemic adverse effects, such as adrenal gland suppression, are common with administration of glucocorticoids.[18,19] Administration of glucocorticoids directly into the lungs via inhalation helps decrease the dose of drug required and therefore decreases adverse effects. Treatment of RAO-affected horses with inhaled fluticasone propionate (2 mg, q 12 h) can decrease neutrophilia in the airways and respiratory efforts without suppressing adrenal gland function.[17,a] Aerosolized beclomethasone dipropionate is also effective for treatment of horses with RAO; however, doses between 0.5 and 1.5 mg twice daily have resulted in substantial adrenal gland suppression.[20] Adrenocortical suppression as a result of inhaled glucocorticoids is attributable in part to absorption of the fraction deposited in the nasopharynx and subsequently swallowed. One way to decrease nasopharyngeal deposition is to actuate the metered dose inhaler through a spacer.[21] Aerosol delivery systems currently available for horses include a spacer.

Incomplete improvement in response to administration of glucocorticoids or environmental changes appears to be related to initial disease severity (ie, the most severely affected horses are less likely to recover normal pulmonary function after treatment). The difficulty in assessing disease severity is that standard pulmonary function tests appear to lack sensitivity and may not detect airway obstruction until it is pronounced and clinically evident.[5,7] We have developed a pulmonary function test that involves use of forced expiration to specifically detect peripheral airway obstruction.[22] Forced expiration is more sensitive and specific for diagnosing RAO than is physical examination and standard pulmonary function tests.[23] Most RAO-affected horses in clinical remission have obstructed airways detectable by use of the forced-expiration method, even though results of physical examination and lung function tests during tidal breathing are normal.

The purpose of the study reported here was to assess whether administration of glucocorticoids would provide additional benefit to environmental modification for the treatment of horses with naturally developing RAO and whether the effect depended on disease severity at the start of treatment. The effects of treatment for 4 weeks with inhaled fluticasone, an inhaled control substance, or oral administration of prednisone on clinical signs, pulmonary function, and results of cytologic evaluation of BALF were evaluated in horses with mild, moderate, and severe RAO that were maintained only on pasture. Additionally, we measured the effects of glucocorticoids on adrenocortical function.

Materials and Methods

Animals—Twenty-eight horses (16 mares, 11 geldings, and 1 stallion) between 10 and 25 years of age were recruited from horses referred to the Purdue University Veterinary Teaching Hospital in response to a mailing sent to practitioners in the area. Inclusion criteria were a history of chronic respiratory disease (coughing or intermittent mucopurulent nasal discharge) for > 2 years, recurrent episodes of increased expiratory efforts when horses were fed hay, and management of the horses had not changed during the preceding 6 months. Historical data obtained also included the amount of time horses spent indoors and outdoors daily and whether hay was part of the diet. Owners signed a consent form after reviewing a document explaining the purpose of the study and the study protocol. An institutional animal care and use committee approved all procedures.

Study design—The study was conducted during 2 winter seasons (winter of 1998 and winter of 1999). The design was a randomized, double-blind, controlled clinical trial. Clinical examination was performed on each horse before pulmonary function testing was conducted. Clinical examinations were performed by a single investigator (CDC) who was unaware of the medical history, test results, and treatment of each horse. A clinical score (range, 0 to 25) was assigned on the basis of a scale described elsewhere.[7] Another investigator (LLC) administered the pulmonary function tests. After initial data were collected, horses were allocated to 1 of 3 categories on the basis of the degree of airway obstruction measured by mean **forced expiratory flow between 75% and 95% of exhaled vital capacity** ($\mathbf{FEF_{75\%–95\%}}$). The 3 categories of airway obstruction were mild ($15 < FEF_{75\%–95\%} \leq 20$ L/s), moderate ($10 < FEF_{75\%–95\%} \leq 15$ L/s), and severe ($FEF_{75\%–95\%} \leq 10$ L/s). Within each category of severity, horses were randomly assigned to 1 of 3 treatment groups (ie, inhaled fluticasone, inhaled control substance, or oral administration of prednisone). Horses were subsequently maintained outdoors on pasture and fed a complete pelleted feed for the remaining 4 weeks of the study. Clinical evaluation and pulmonary function tests were repeated 2 and 4 weeks after initiation of treatment.

Some horses remained at Purdue University for the duration of the study, whereas the others were discharged to their owners with instructions for treatment. Owners that provided treatment of horses were trained to administer the drugs. They were given a diary in which to record on a daily basis the amount of drug administered and any comments pertaining to adverse effects or problems encountered during treatment administration. A single investigator (LLC) administered treatments to horses that remained at Purdue University for the duration of the study.

Canisters for inhaled drugs were assigned a code. Thus, content of the canisters was unknown to the people administering treatments and was revealed only at the end of the study. Treatments consisted of aerosolized fluticasone propionate (1,980 µg [ie, 9 puffs], q 12 h, for 2 weeks; followed by 1,100 µg [ie, 5 puffs], q 24 h, for 1 week; and then followed by 1,100 µg, q 48 h, for 1 week), inhaled control substance (9 puffs, q 12 h, for 2 weeks; followed by 5 puffs, q 24 h, for

1 week; and then followed by 5 puffs, q 48 h, for 1 week), or oral administration of prednisone (500 mg, q 12 h, for 2 weeks; followed by 200 mg, q 24 h, for 1 week; and then followed by 200 mg, q 48 h, for 1 week). Aerosols were administered by use of a commercially available delivery system.[b] Prednisone tablets were crushed, mixed with approximately 10 mL of a combination of corn syrup and molasses, and administered directly into a horse's mouth.

Pulmonary function tests—Horses were restrained in stocks without sedation, and standard lung function tests were conducted. Esophageal pressure was measured by use of a balloon catheter (inside diameter, 4.8 mm; outside diameter, 6.4 mm; length, 240 cm) with its proximal end connected to a pressure transducer.[c] The esophageal balloon was advanced to the midthorax and used to estimate pleural pressure. A second catheter (inside diameter, 4.8 mm; outside diameter, 6.4 mm; length, 240 cm) connected the other port of the pressure transducer to a mask that was placed tightly around the nose of each horse. **Transpulmonary pressure (ΔP_L)** was defined as the difference between pressure in the mask and the esophageal pressure. Airflow was measured by use of a pneumotachometer[d] fitted to the mask and coupled with a pressure transducer.[e] Output signals from both pressure transducers were recorded by use of computer software.[f] The R_L was computed by use of the isovolume 50% method, and C_{dyn} was computed by dividing tidal volume by the difference in ΔP_L between points of zero flow. Signals from pressure and flow transducers were phase-matched. Pneumotachograph calibration was performed with a 3-L calibrated syringe,[g] and the transducer used to measure ΔP_L was calibrated with a water manometer.[h]

Forced expiration testing was conducted in accordance with a procedure that has been completely described and validated.[22] The face mask was removed after collection of standard pulmonary function data, but the esophageal catheter and associated transducer were left in place to measure ΔP_L, as described previously. Each horse was sedated by IV administration of a combination of detomidine (0.03 mg/kg) and butorphanol tartrate (0.02 mg/kg). Four minutes after injection of the detomidine-butorphanol, a cuffed nasotracheal tube (inside diameter, 20 mm) was advanced through the nostrils and nasal passages to the proximal third of the trachea. The cuff was inflated to prevent air leaks. The proximal end of the nasotracheal tube was connected to a 3-way valve with ports to a ventilator[i] or a solenoid valve connected to a 1,433-L negative-pressure reservoir that was pumped down to –220 cm H_2O.

Initially, the solenoid valve was in the closed position. Each horse was hyperventilated with the ventilator to decrease respiratory drive (minute ventilation, 48 to 55 L/min). The 3-way valve was turned in the direction of the pressure reservoir. Then, the lungs were inflated to total lung capacity (ΔP_L, 30 cm H_2O) by use of a pressurized tank (150 to 190 cm H_2O). Finally, the computer-driven solenoid valve opened suddenly, exposing airways to the negative-pressure reservoir and inducing forced expiration. During expiration, the instantaneous pressure change was measured within the reservoir. **Expired lung volume (dV_L)** was calculated by use of the following equation:

$$dV_L = 845.3 \times (dP_r/P_r),$$

where dP_r is the instantaneous pressure change, and P_r is the initial pressure in the reservoir. The instantaneous flow rate was calculated as the expired volume per sampling interval. Flow and volume data were combined to generate forced expiratory flow-versus-volume curves. Analysis of the flow-versus-volume curve yielded **forced vital capacity (FVC)**, **forced expiratory volume in 1.5 seconds ($FEV_{1.5}$)**, **forced expiratory flow after 95% of FVC has been exhaled ($FEF_{95\%}$)**, and $FEF_{75\%–95\%}$. The procedure was repeated until 3 acceptable and reproducible curves were obtained. Forced expiration variables were measured from the curve that yielded the largest values for FVC and $FEV_{1.5}$.

Cytologic evaluation of BALF—A flexible videoendoscope (diameter, 9 mm; length, 200 cm) was passed through the nostrils and nasal passages and advanced until wedged into the caudodorsal airways. Coughing was alleviated by spraying airways with a 0.2% lidocaine solution as the endoscope was advanced into the respiratory tract. A 250-mL bolus of sterile saline (0.9% NaCl) solution was infused under pressure through the endoscope biopsy channel. The BALF was immediately aspirated by use of a suction pump, and harvested BALF was placed on ice. Samples of BALF were processed within 20 minutes after collection. Total nucleated cell count was determined manually by use of a hemacytometer. Cytologic specimens were prepared by centrifugation and processed with Wright's stain. Differential cell counts were determined by examination of approximately 500 leukocytes/slide. Clinical pathologists (DBD and SPC) who examined the BALF were unaware of the category and treatment group for each horse.

Evaluation of adrenocortical function—Adrenocortical function was assessed by measuring serum cortisol and adrenocortical responsiveness before and 2 and 4 weeks after initiation of treatment. Adrenocortical testing was performed only on horses that remained at Purdue University for the duration of the study; 10 horses were randomly selected to undergo adrenocortical testing. Adrenocortical tests were conducted the day before pulmonary function testing. On test days, an initial blood sample was collected at 8 AM and used for measurement of the serum cortisol concentration (baseline value). Then, 100 units of cosyntropin was injected IV. A second blood sample was collected 2 hours after cosyntropin administration for quantification of serum cortisol concentration.[24] After harvest, serum samples were stored at –20°C until cortisol concentrations were analyzed. Serum cortisol concentrations were quantified by use of chemiluminescent enzyme immunoassay within 1 week after sample collection.

Statistical analyses—Effects of treatment (fluticasone, prednisone, or control), treatment location (home or Purdue University), time (before treatment, 2 weeks after initiation of treatment, and 4 weeks after initiation of treatment), and the treatment-by-time interaction on clinical score, pulmonary function, baseline serum cortisol concentration, and percentage change in serum cortisol content after cosyntropin administration were tested by use of a multivariate ANOVA with commercially available software.[25] When a significant effect of time or the time-by-treatment interaction was detected, differences of least-squares means were calculated for paired comparisons and *P* values adjusted by use of the Tukey-Kramer procedure. Data from cytologic evaluation of BALF were logarithmically transformed, which yielded a normal distribution. The effects of treatment and time on results of cytologic evaluation of BALF were evaluated as described previously. Associations between disease severity before initiation of treatment and housing or feeding practices prior to the study were tested by use of χ^2 analysis.[26] Results were expressed as mean ± SD. Significance was set at values of $P \leq 0.05$.

Results

Animals—Thirteen of 28 (46%) horses remained at Purdue University for the duration of the study,

which included 3 that received inhaled fluticasone, 6 that received the inhaled control substance, and 4 that received orally administered prednisone. Fifteen (54%) horses were treated at home by the owners, which included 7 that received inhaled fluticasone, 3 that received the inhaled control substance, and 5 that received orally administered prednisone. Among horses with mild airflow obstruction, 3 were treated with inhaled fluticasone, 3 with the inhaled control substance, and 3 with orally administered prednisone. Among horses with moderate airflow obstruction, 4 were treated with inhaled fluticasone, 2 with the inhaled control substance, and 1 with orally administered prednisone. Finally, among horses with severe airflow obstruction, 3 were treated with inhaled fluticasone, 4 with the inhaled control substance, and 5 with orally administered prednisone. Treatment was tolerated well in all horses, and no adverse effects were observed.

Housing and treatment location—We did not detect a significant (P = 0.25) association between housing conditions (ie, indoors vs outdoors) prior to the start of the study and severity of airflow obstruction before initiation of treatment. The proportion of horses with severe obstruction that were fed hay prior to the study (9/10 [90%]) was significantly (P = 0.01) higher than the proportion of horses with mild obstruction that were fed hay prior to the study (2/7 [28%]).

We did not detect a significant (P = 0.11) interaction between location of the horse during treatment (Purdue University or owner's farm) and treatment effect on clinical score and results of pulmonary function tests.

Clinical score—Before treatment initiation, mean ± SD score of horses with severe airway obstruction (13.8 ± 6.4) was significantly (P = 0.014) higher than that of horses with mild airway obstruction (6.3 ± 5.1). Clinical score of horses with moderate airway obstruction (12.0 ± 8.0) did not differ significantly from scores for horses with mild or severe airway obstruction. There was significant (P < 0.001) improvement in the clinical score over time in all horses regardless of treatment (**Figure 1**). The largest effect was in horses with severe airway obstruction, which improved significantly (P = 0.003) after treatment was administered for 2 weeks (8.2 ± 4.6) and 4 weeks (4.9 ± 3.5), respectively. There was no significant difference in clinical score among the 3 treatments for horses with mild, moderate, and severe airway obstruction. At the end of the 4-week treatment period, clinical scores of horses receiving orally administered and inhaled glucocorticoids were significantly (P = 0.01) lower than clinical scores determined before initiation of treatment. However, clinical scores of horses treated with the inhaled control substance did not improve significantly (P = 0.13) from the clinical scores before initiation of treatment.

Standard lung function tests—Before initiation of treatment, horses with severe airway obstruction had significantly (P = 0.01) higher ΔP_{Lmax} and R_L than horses with mild or moderate obstruction (**Table 1**). In addition, C_{dyn} was significantly (P = 0.01) lower in horses with severe airway obstruction, compared with values in other horses. Lung function of horses with mild or moderate airway obstruction did not differ significantly (P = 0.07).

We detected a significant (P = 0.002) decrease in ΔP_{Lmax} during the 4-week period of environmental modification and treatment in all horses (**Figure 2**). However, values did not differ significantly (P = 0.51) among the 3 treatments. No further decrease in ΔP_{Lmax} was detected after the first 2 weeks of treatment. When horses were grouped on the basis of degree of airway obstruction, the decrease in ΔP_{Lmax} over time was only significant (P < 0.001) in severely obstructed horses. Horses with severe RAO treated with inhaled fluticasone had the highest ΔP_{Lmax} before the initiation of treatment, and they had a significantly (P = 0.004) greater decrease in ΔP_{Lmax}, compared with values for horses treated with orally administered prednisone and the inhaled control substance, during the first 2 weeks of treatment (**Figure 3**). No further benefit was detect-

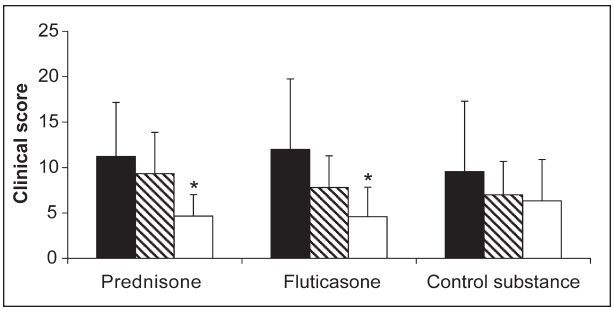

Figure 1—Mean ± SD clinical score of 28 horses with recurrent airway obstruction (RAO) before initiation of treatment (black bars) and after placement in a low-dust environment (ie, pasture with pelleted feed) and treatment (orally administered prednisone [n = 9], inhaled fluticasone [10], or an inhaled control substance [9]) for 2 (diagonal-striped bars) and 4 (white bars) weeks. *Within a treatment group, value differs significantly (P < 0.05) from the value before initiation of treatment.

Table 1—Pulmonary function in horses with recurrent airway obstruction (RAO) grouped on the basis of degree of airway obstruction (mild, moderate, or severe) before initiation of treatment.

Pulmonary function	Mild (9)	Moderate (7)	Severe (12)
ΔP_{Lmax} (cm H_2O)	8.5 ± 3.7	11.9 ± 7.1	33.9 ± 19.7*,†
R_L (cm H_2O/L/s)	0.60 ± 0.39	0.85 ± 0.39	2.18 ± 1.31*,†
C_{dyn} (L/cm H_2O)	2.84 ± 1.28	1.94 ± 1.04	0.75 ± 0.59*,†
FVC (L)	42.4 ± 5.4	39.7 ± 5.2	29.9 ± 7.0*,†
$FEV_{1.5}$ (L)	40.0 ± 3.9	35.1 ± 3.8	24.4 ± 6.3*,†
$FEV_{1.5}$-to-FVC ratio	0.946 ± 0.036	0.885 ± 0.034*	0.813 ± 0.052*,†
$FEF_{75\%-95\%}$ (L/s)	17.6 ± 1.3	12.6 ± 1.8*	5.1 ± 1.7*,†
$FEF_{95\%}$ (L/s)	7.4 ± 5.0	2.5 ± 0.5*	2.2 ± 0.6*

Numbers in parentheses are number of horses.

*Within a row, value differs significantly (P = 0.01) from the value for horses with mild airway obstruction. †Within a row, value differs significantly (P = 0.01) from the value for horses with moderate airway obstruction.

ΔP_{Lmax} = Maximum change in transpulmonary pressure. R_L = Pulmonary resistance. C_{dyn} = Dynamic compliance. FVC = Forced vital capacity. $FEV_{1.5}$ = Forced expiratory volume in 1.5 seconds. $FEF_{75\%-95\%}$ = Forced expiratory flow between 75% and 95% of exhaled vital capacity. $FEF_{95\%}$ = Forced expiratory flow after 95% of FVC has been exhaled.

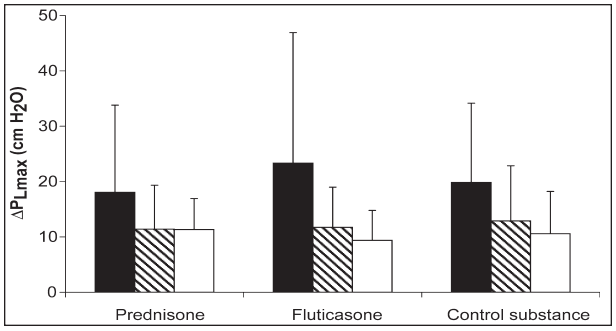

Figure 2—Mean ± SD changes in maximum transpulmonary pressure (ΔP_{Lmax}) in RAO-affected horses before initiation of treatment (black bars) and after placement in a low-dust environment (ie, pasture with pelleted feed) and treatment (orally administered prednisone, inhaled fluticasone, or an inhaled control substance) for 2 (diagonal-striped bars) and 4 (white bars) weeks.

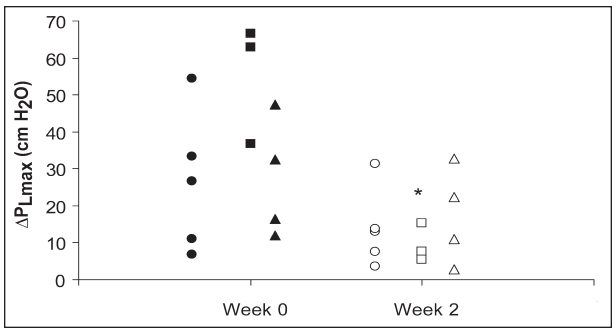

Figure 3—The ΔP_{Lmax} in horses with severe RAO before (black symbols) and after (white symbols) placement in a low-dust environment (ie, pasture with pelleted feed) and treatment with orally administered prednisone (circles; n = 5), inhaled fluticasone (squares; 3), or an inhaled control substance (triangles; 4) for 2 weeks. *Within a treatment group, value differs significantly ($P = 0.004$) from the value before initiation of treatment.

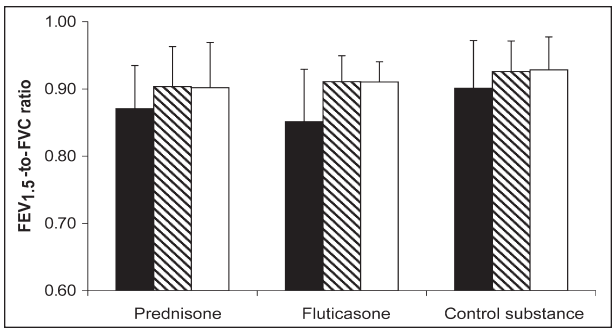

Figure 4—Mean ± SD forced expiratory volume in 1.5 seconds-to-forced vital capacity ($FEV_{1.5}$-to-FVC) ratio in horses with severe RAO before initiation of treatment (black bars) and after placement in a low-dust environment (ie, pasture with pelleted feed) and treatment with orally administered prednisone, inhaled fluticasone, or an inhaled control substance for 2 (diagonal-striped bars) and 4 (white bars) weeks.

ed during the final 2 weeks of treatment. At the end of the 4-week treatment period, ΔP_{Lmax} did not differ significantly ($P = 0.7$) among the 3 treatment groups.

Over the 4-week treatment period, R_L decreased in all horses regardless of treatment. Horses with severe airway obstruction had a significant ($P < 0.001$) decrease in R_L after treatment for 2 weeks, and that

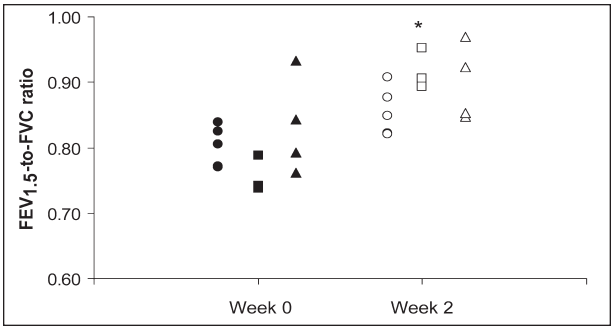

Figure 5—The $FEV_{1.5}$-to-FVC ratio in horses with severe RAO before (black symbols) and after (white symbols) placement in a low-dust environment (ie, pasture with pelleted feed) and treatment with orally administered prednisone (circles; n = 5), inhaled fluticasone (squares; 3), or an inhaled control substance (triangles; 4) for 2 weeks. *Within a treatment group, value differs significantly ($P < 0.001$) from the value before initiation of treatment.

improvement was maintained through 4 weeks of treatment. Similar to ΔP_{Lmax}, severely obstructed horses treated with inhaled fluticasone (mean ± SD R_L was 3.17 ± 1.0 cm H_2O/L/s before initiation of treatment and 0.63 ± 0.49 cm H_2O/L/s after receiving treatment for 2 weeks) had a significantly ($P = 0.02$) greater decrease in R_L than horses treated with orally administered prednisone (mean R_L was 1.60 ± 0.92 cm H_2O/L/s before initiation of treatment and 1.10 ± 0.76 cm H_2O/L/s after receiving treatment for 2 weeks) and the inhaled control substance (mean R_L was 2.17 ± 1.71 cm H_2O/L/s before initiation of treatment and 1.18 ± 0.81 cm H_2O/L/s after receiving treatment for 2 weeks) during the first 2 weeks of treatment.

When data for all horses were combined, C_{dyn} did not change significantly ($P = 0.44$) after horses received treatment for 2 or 4 weeks. Again, C_{dyn} of severely obstructed horses increased significantly ($P = 0.01$) after horses received treatment for 2 weeks, and the improvement persisted at 4 weeks of treatment. However, no significant ($P = 0.86$) effect of treatment on C_{dyn} was detected.

We did not detect a significant difference between horses treated with orally administered prednisone and the inhaled control substance for any of the variables during standard pulmonary function tests. At the end of the 4-week treatment period, ΔP_{Lmax} and R_L were not significantly ($P = 0.09$) different among horses that initially had mild, moderate, and severe airflow obstruction. However, mean ± SD C_{dyn} of severely obstructed horses (1.46 ± 0.86 L/cm H_2O) after treatment for 4 weeks was significantly ($P = 0.035$) lower than mean C_{dyn} in horses with mild obstruction (2.63 ± 1.09 L/cm H_2O).

Forced expiration tests—Before initiation of treatment, horses with severe airway obstruction had a significantly ($P = 0.01$) lower FVC and $FEV_{1.5}$, compared with values for horses with mild or moderate airway obstruction (Table 1). The $FEF_{95\%}$ was significantly ($P < 0.001$) lower in horses with moderate or severe airway obstruction, compared with the value in horses with mild obstruction. In all RAO-affected horses, FVC (36.4 ± 8.2 L) and $FEV_{1.5}$ (32.1 ± 8.5 L) before initia-

tion of treatment were significantly ($P = 0.03$) lower than values after treatment for 2 weeks (FVC, 39.5 ± 7.0 L; $FEV_{1.5}$, 36.0 ± 6.3 L) and 4 weeks (FVC, 40.1 ± 6.4 L; $FEV_{1.5}$, 36.6 ± 5.8 L). Overall, the $FEV_{1.5}$-to-FVC ratio increased significantly ($P < 0.001$) during the treatment period (**Figure 4**). However, the ratio did not differ significantly ($P = 0.53$) among treatments. The greatest response to treatment was during the first 2 weeks of treatment with no further improvement detected between weeks 2 and 4 of treatment.

When data were analyzed on the basis of degree of airway obstruction (mild, moderate, or severe), only horses with severe airway obstruction had a significant ($P < 0.001$) increase in FVC and $FEV_{1.5}$ after treatment for 2 weeks (FVC, 29.9 ± 7.0 L before initiation of treatment and 40.3 ± 5.9 L after treatment for 2 weeks; $FEV_{1.5}$, 24.4 ± 6.3 L before initiation of treatment and 35.8 ± 5.1 L after treatment for 2 weeks) and the improvement was similar among the 3 treatment groups. There was no further improvement in FVC and $FEV_{1.5}$ between the second and fourth week of treatment (FCV after treatment for 4 weeks, 39.6 ± 6.2 L; $FEV_{1.5}$ after treatment for 4 weeks, 35.2 ± 6.0 L). Horses with severe RAO treated by use of inhaled fluticasone were the only group with a significant ($P < 0.001$) increase in the $FEV_{1.5}$-to-FVC ratio, compared with values for horses with severe RAO treated by use of orally administered prednisone or the inhaled control substance, during the first 2 weeks of treatment (**Figure 5**). The improvement was maintained after 4 weeks of treatment. After receiving treatment for 4 weeks, the $FEV_{1.5}$-to-FVC ratio did not differ significantly among the 3 treatment groups.

Similarly, $FEF_{75\%–95\%}$ increased significantly ($P < 0.001$) over the course of treatment regardless of the treatment administered (before initiation of treatment, 11.0 ± 5.7 L/s; after treatment for 2 weeks, 13.9 ± 4.8 L/s; and after treatment for 4 weeks, 15.2 ± 5.2 L/s), and the effect was attributed primarily to a significant ($P < 0.001$) improvement for horses with severe airway obstruction. Horses with mild or moderate obstruction did not have a significant ($P = 0.35$) change in $FEF_{75\%–95\%}$ over time. There was a significant ($P = 0.03$) effect of treatment on $FEF_{75\%–95\%}$ for horses with severe RAO during the first 2 weeks of treatment with inhaled fluticasone (3.3 ± 0.1 L before initiation of treatment and 15.2 ± 0.4 L/s after treatment for 2 weeks), compared with values for orally administered prednisone (5.1 ± 1.3 L/s before initiation of treatment and 9.2 ± 2.8 L/s after treatment for 2 weeks) or the inhaled control substance (6.5 ± 1.6 L/s before initiation of treatment and 13.0 ± 5.1 L/s after treatment for 2 weeks). After treatment for 4 weeks, $FEF_{75\%–95\%}$ did not differ significantly

Table 2—Median (minimum–maximum) values for cytologic evaluation of bronchoalveolar fluid obtained from horses with RAO grouped on the basis of severity of airway obstruction (mild, moderate, or severe) before initiation of treatment.

	Mild (n = 9)		Moderate (6)		Severe (12)	
Variable	**No.**	**%**	**No.**	**%**	**No.**	**%**
Volume (mL)	125 (50–150)	NA	75 (50–130)	NA	90 (30–150)	NA
Total nucleated cells (No./µL)	253 (80–414)	NA	255 (117–3,564)	NA	286 (98–913)	NA
Neutrophils (No./µL)	46 (15–87)	17 (7–67)	60 (18–3,278)	17 (12–92)	75 (19–292)	25 (9–85)
Lymphocytes (No./µL)	91 (25–295)	43 (19–71)	120 (49–893)	43 (6–60)	61 (20–621)	31 (7–68)
Macrophages (No./µL)	68 (13–137)	28 (10–47)	63 (14–307)	19 (3–33)	94 (18–220)	34 (6–49)
Eosinophils (No./µL)	0 (0–1)	0 (0–1)	3 (0–55)[a]	1 (0–32)	0 (0–2)[b]	0 (0–1)
Mast cells (No./µL)	4 (0–5)	1 (0–3)	4 (0–6)	1 (0–4)	2 (0–31)	1 (0–10)
Epithelial cells (No./µL)	1 (0–5)	1 (0–7)	3 (0–9)	1 (0–4)	5 (0–17)	2 (0–6)

NA = Not applicable.
[a,b]Numbers with different superscript letters differ significantly ($P < 0.05$).

Table 3–Median (minimum–maximum) values for cytologic evaluation of bronchoalveolar fluid obtained from horses with RAO before (week 0) and 2 and 4 weeks after placement in a low-dust environment (ie, pasture with pelleted feed) and treatment with orally administered prednisone, inhaled fluticasone, or an inhaled control substance.

	Prednisone			Fluticasone			Control substance		
Cell types	**Week 0**	**Week 2**	**Week 4**	**Week 0**	**Week 2**	**Week 4**	**Week 0**	**Week 2**	**Week 4**
Neutrophils (No./µL)	87 (15–3,278)	98 (8–259)	69 (20–156)	62 (18–292)	47 (0–232)	66 (13–169)	52 (19–104)	38 (2–207)	36 (16–76)
Lymphocytes (No./µL)	58 (20–621)	105 (9–655)	135 (21–1,053)	85 (49–893)	145 (19–349)	156 (52–283)	78 (32–295)	123 (47–154)	85 (51–238)
Macrophages (No./µL)	50 (13–220)	78 (4–450)	32 (7–330)	83 (31–307)	115 (31–325)	105 (16–255)	98 (14–137)	114 (49–394)	97 (45–267)
Eosinophils (No./µL)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–17)	0 (0–76)	0 (0–6)	0 (0–55)	0 (0–7)	0 (0–39)
Mast cells (No./µL)	2 (0–8)	4 (0–11)	3 (2–7)	4 (0–6)	4 (1–8)	3 (0–11)	3 (1–31)	6 (1–13)	3 (0–41)

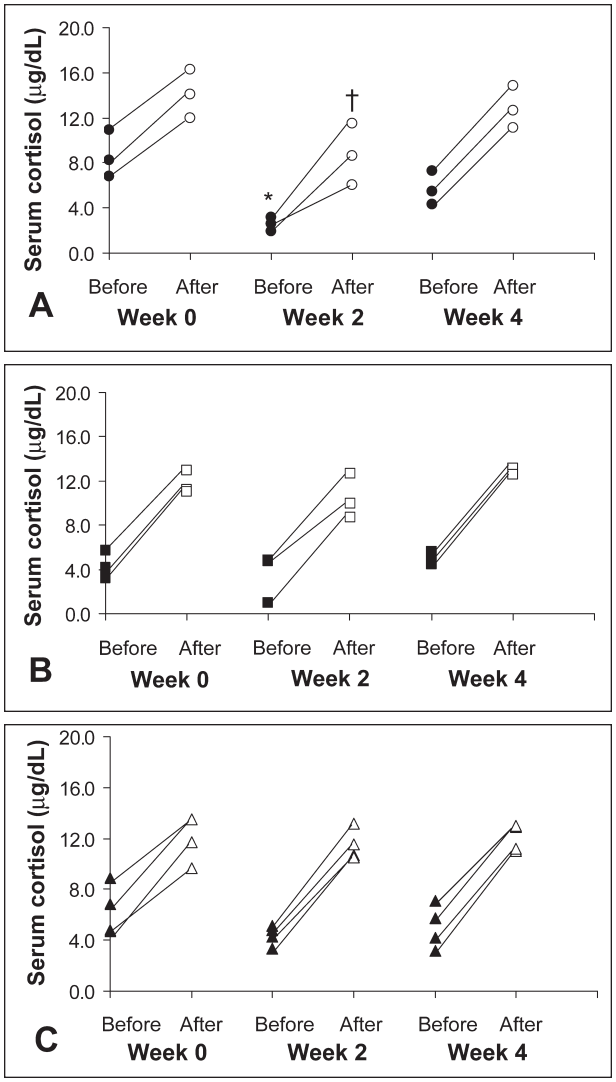

Figure 6—Serum cortisol concentrations before (black symbols) and after (white symbols) administration of cosyntropin to horses with severe RAO before (week 0) and 2 and 4 weeks after placement in a low-dust environment (ie, pasture with pelleted feed) and treatment with orally administered prednisone (circles; A), inhaled fluticasone (squares; B), or an inhaled control substance (triangles; C). *Value differs significantly ($P = 0.04$) from the value before administration of cosyntropin at week 0. †Value differs significantly ($P = 0.027$) from the value after administration of cosyntropin at week 0.

($P = 0.18$) among the 3 treatment groups. There was also no significant ($P = 0.3$) effect of time or treatment on $FEF_{95\%}$.

Evaluation of BALF—Fluid volume recovered after the bronchoalveolar lavage procedure, total nucleated cell counts, absolute cell counts, and percentages of cells were not significantly different among the 3 treatment groups before initiation of treatment, except that eosinophil counts were significantly higher in horses with moderate airway obstruction, compared with counts in horses with severe airway obstruction (**Table 2**).

Mean ± SD volume of fluid recovered before initiation of treatment (91.1 ± 35.6 mL) was significantly ($P = 0.023$) lower than the volume recovered after horses were treated for 2 (105.2 ± 33.2 mL) or 4 (106.4 ± 44.3 mL) weeks. However, treatment did not significantly ($P = 0.55$) affect amount of recovered BALF.

We did not detect a significant effect of time ($P = 0.07$) or treatment ($P = 0.25$) on any of the cytologic variables for BALF expressed as absolute cell counts or percentages (**Table 3**). The analysis was repeated separately for each horse with mild, moderate, or severe airway obstruction, and no significant effects of time or treatment on any of the cytologic variables of BALF expressed as absolute cell counts or percentages were detected. One horse with severe airway obstruction had a markedly increased total nucleated cell count before initiation of treatment (3,564 cells/µL) because of a high percentage of neutrophils (92%).

Adrenocortical function—Adrenocortical function was tested in 10 horses, including 3 treated with inhaled fluticasone, 4 treated with the inhaled control substance, and 3 treated with orally administered prednisone. Baseline serum cortisol concentration decreased significantly ($P = 0.04$) after 2 weeks of treatment with orally administered prednisone, compared with the baseline concentration after 2 weeks of treatment with inhaled fluticasone or the control substance (**Figure 6**). There was no significant ($P = 0.7$) difference in baseline serum cortisol concentration among the 3 treatment groups after 4 weeks of treatment.

Serum cortisol concentration after cosyntropin administration was significantly ($P = 0.027$) lower in RAO-affected horses after oral administration of prednisone for 2 weeks, compared with concentrations for RAO-affected horses treated for 2 weeks with inhaled fluticasone or the control substance (Figure 6). There was no significant ($P = 0.7$) difference in serum cortisol concentration after cosyntropin administration among the 3 treatment groups after 4 weeks of treatment. There was no significant ($P = 0.1$) effect of time or treatment on the relative increase in serum cortisol concentration after cosyntropin administration.

Discussion

Clinical signs and pulmonary function of RAO-affected horses improved significantly within 2 weeks after horses were placed in a low-dust environment and received treatment with inhaled fluticasone, orally administered prednisone, or an inhaled control substance. The improvement persisted for an additional 2 weeks, during which drug dosages were gradually decreased. Overall, horses treated with either of the glucocorticoids or the control substance had the same degree of clinical and functional improvement after 4 weeks of treatment, suggesting that improvement was primarily a result of environmental changes rather than medical treatment. Placement in a low-dust environment and treatment with inhaled fluticasone, orally administered prednisone, or the control substance for 4 weeks did not have a significant effect on results of cytologic evaluation of BALF obtained from horses with RAO. Analysis of results of adrenocortical function tests indicated that prednisone resulted in a significant suppression of cortisol secretion after oral administration for 2 weeks at a dose of 500 mg twice daily. Typical cortisol secretion recovered following 2 additional weeks of

treatment with decreasing doses of prednisone. Inhaled fluticasone and an inhaled control substance did not affect adrenocortical function during the 4-week study period. When RAO-affected horses were grouped on the basis of the degree of airway obstruction, clinical and functional improvement was only significant in horses that initially had severe airway obstruction. In this group of horses, inhaled fluticasone resulted in a more pronounced improvement in pulmonary function, compared with results for prednisone or the control substance, after the first 2 weeks of treatment.

A potential source of bias in the study reported here was the fact that approximately half of the RAO-affected horses were treated by the owners. Despite the fact that each owner was instructed on how to administer treatments and practiced treatment administration under the supervision of the investigators, treatments may have been improperly administered. Horses were all maintained outdoors; however, exposure to aeroallergens may have differed among horses housed at their home facilities, compared with exposure for horses maintained at Purdue University. The study was conducted during 2 consecutive winter seasons when aeroallergens are at the lowest concentrations in an attempt to decrease the potential impact of such exposure. The inclusion of treatment location (ie, Purdue University vs owner's facility) as a covariate in the statistical analysis did not change the findings, which suggested that these uncontrolled factors did not confound the study results.

Hay feeding was significantly associated with severe airflow obstruction before initiation of treatment. Reports[7,27,28] support the role of exposure to hay dust, especially molds, in triggering clinical signs and pulmonary dysfunction in horses susceptible to RAO. Removal of hay from the environment, even when a horse is housed indoors, usually leads to substantial clinical and functional improvement.[15,29] We observed the same effect in horses with severe airflow obstruction after they were placed on pasture and fed a complete pelleted feed, regardless of whether they were treated with a glucocorticoid. These findings corroborate the conclusions of another study[15] with regard to the fact that oral administration of prednisone does not provide additional benefit to environmental changes in horses with RAO. However, the magnitude of the effect of environmental changes on the measured variables may have masked a potential treatment effect attributable to oral administration of prednisone. Horses with mild or moderate airflow obstruction had a less dramatic change in environment because most were already maintained on pasture and not fed hay prior to the start of the study. Nevertheless, mildly and moderately affected horses had no significant pulmonary function changes during the 4-week course of treatment. Therefore, even when RAO-affected horses were maintained in a low-dust environment, an effect of prednisone treatment on clinical signs or pulmonary function was not detected. This lack of effect is likely the result of poor oral bioavailability of prednisone.[16]

The lack of effect of inhaled fluticasone on cytologic variables in BALF was unexpected because another study[17] revealed a significant decrease in the number of neutrophils in BALF of RAO-affected horses after 3 weeks of treatment with the same dose of fluticasone and an identical aerosol delivery system to that used in the study reported here. Similar suppression of airway neutrophilia in RAO-affected horses maintained in a moldy hay environment after treatment with inhaled beclomethasone for 7 days has been reported.[11] However, both of those studies used horses that had been in disease remission as a result of maintaining them on pasture for several months before the beginning of the studies. In addition, glucocorticoid treatment was initiated within 5 to 7 days after exposing RAO-affected horses to a moldy hay environment. Horses enrolled in our study had clinical signs of RAO for at least 2 years before onset of the study, and 16 of 24 (67%) were fed hay on a daily basis during that time. This prolonged exposure to inhaled dust particles prior to the study reported here may have led to more severe airway obstruction, which could have resulted in lower drug deposition into the lungs and poor response to treatment.[30] In fact, horses enrolled in our study had values for ΔP_{Lmax} (mean ± SD, 20 ± 3 cm H_2O) that were not higher than those for horses in the other studies (mean range, 32 to 42 cm H_2O[11] and 25 to 40 cm H_2O[17]), which suggested that the degree of airway obstruction in our RAO-affected horses was comparatively less. Furthermore, our horses had a significant improvement in pulmonary function after treatment with inhaled fluticasone for 2 weeks. Alternatively, the dose of fluticasone may have been too small to reverse the effect of long-term exposure to inhaled dust particles, which resulted in overexpression of inflammatory mediators and invasion of the lung parenchyma by inflammatory cells. The dose of glucocorticoids required to improve pulmonary function may have been smaller than the dose needed to decrease airway inflammation.

Pulmonary dysfunction and airway neutrophilia are evident within 4 to 5 hours after RAO-affected horses are exposed to moldy hay.[31] Pulmonary function returns to normal after affected horses are placed in a low-dust environment; however, the neutrophil count in BALF may remain increased for prolonged periods.[32] Some RAO-affected horses treated with aerosolized or systemically administered glucocorticoids may improve clinically and functionally without detectable changes in BALF neutrophil counts.[11,12,19] This apparent lack of correlation between airway inflammatory cells and pulmonary function in response to glucocorticoids may result from factors involved in balancing recruitment and removal of inflammatory cells in the lungs. In humans with asthma, recruitment of leukocytes to the lungs and their activation are controlled by chemotactic cytokines released by airway epithelial cells and macrophages,[33] some of which have also been identified in horses with heaves.[17,34] Apoptosis allows safe removal of inflammatory cells; however, programmed cell death may be inhibited by various inflammatory mediators or stimulated by glucocorticoids. Lung granulocytes from RAO-affected horses fed moldy hay also had delayed apoptosis, which may explain the persistent airway neutrophilia in horses that are maintained in such an environment.[35] However, glucocorticoids

inhibit apoptosis of human neutrophils. Whether glucocorticoids have similar effects on equine neutrophils is currently unknown.

Inhaled beclomethasone dipropionate can effectively control clinical signs and pulmonary dysfunction in horses with RAO.[13,14] However, one of the adverse effects is adrenocortical suppression with doses as low as 500 µg twice daily.[18,20] Interestingly, aerosolized treatments in horses with heaves by use of 500 µg of beclomethasone has provided similar improvement in lung function and clinical signs as doses up to 1,500 µg while inducing minimal adrenocortical suppression.[20] Analysis of the results of our study revealed that administration of 1,980 µg of fluticasone twice daily for 2 weeks did not suppress adrenocortical function. These findings are in agreement with those in another report.[a] Adrenocortical suppression and other systemic effects of inhaled glucocorticoids depend on pharmacokinetics of the drug. In humans, up to 80% of an actuation from a metered-dose inhaler is deposited in the oropharynx and swallowed and only approximately 20% is deposited in the small airways.[21] Drug deposition in the oropharynx may be decreased by adding a spacer between the metered dose inhaler and patient. The swallowed portion of the drug is metabolized in the same manner as an orally administered formulation, and the fraction reaching the airways is absorbed in the bloodstream and metabolized in the same manner as for a dose administered IV.

To minimize systemic adverse effects of glucocorticoids, new inhaled drugs have been developed to achieve low oral absorption and extensive first-pass metabolism by the liver. In humans, inhalation of fluticasone propionate results in < 1% of swallowed drug being absorbed and almost complete first-pass metabolism by the liver.[21] In comparison, oral bioavailability of inhaled beclomethasone dipropionate is approximately 20%, and its first-pass metabolism by the liver is less complete than for fluticasone propionate. As a result, suppression of adrenal gland function by inhaled fluticasone is less than that for beclomethasone.[36] Metabolism of inhaled glucocorticoids in horses is currently unknown. However, these mechanisms may explain the lack of adrenocortical suppression with fluticasone, compared with that for beclomethasone. Also, nasopharyngeal deposition of inhaled glucocorticoids may be more important with the hand-held device used for beclomethasone administration than with the mask system coupled to a spacer that was used for fluticasone administration.

Oral administration of prednisone resulted in significant suppression of serum cortisol concentrations, despite the fact that it is poorly absorbed in horses.[16] Progressive reduction of the daily dose and alternate-day administration during a 2-week period resulted in the recovery of normal function of the hypothalamopituitary-adrenal axis. Adrenal suppression is likely to be minimal and short-lived after administration of glucocorticoids for only a few days.[18] However, treatment lasting weeks or months with an adrenal suppressive dose of glucocorticoids requires gradual withdrawal to avoid clinical signs of adrenal gland insufficiency. Alternate-day treatment should be initiated with a short-acting glucocorticoid (eg, prednisone, prednisolone, or methylprednisolone) administered once in the morning to allow stimulation of endogenous glucocorticoid synthesis during the day of no treatment.[37] A typical adrenal gland response after cosyntropin administration indicates recovery of adrenocortical function. In our study, cortisol response to administration of cosyntropin was unchanged after treatment with a high dose of prednisone for 2 weeks, which suggested adequate adrenal gland reserves.

Treatment of horses with long-standing RAO by use of a combination of a low-dust environment and administration of glucocorticoids or an inhaled control substance for 4 weeks resulted in improvements in clinical signs and pulmonary function. A beneficial effect of inhaled fluticasone on pulmonary function was only detected in horses with severe airway obstruction after administration of 1,980 µg twice daily for the first 2 weeks of treatment. As a result of environmental changes, pulmonary function of horses treated with an inhaled control substance also improved significantly during the same 2-week period. However, these benefits were not accompanied by an appreciable decrease in airway inflammation. Analysis of our results supports the early use of inhaled fluticasone in conjunction with environmental management for the treatment of horses with severe RAO. However, the control of RAO should focus primarily on environmental management, especially the removal of hay from the diet.

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