Pferdeheilkunde 17 (2001) 5 (September/Oktober) 471–476

Pulmonary function at rest in show jumpers, event and endurance horses assessed by indices derived from the single breath diagram for CO_2 (SBD-CO_2)

Conny Herholz[1], *R. Straub*[1], *A. Imhof* [1], *S. Lüthi* [1], *D. Burger* [2] and *A. Busato*[3]

[1] Department of Equine Internal Medicine, Faculty of Veterinary Medicine, University of Berne, Switzerland, [2] Haras Federal, Avenches, Switzerland,
[3] Maurice E. Müller Center for Continuing Education and Documentation for Orthopaedic Surgery, Berne, Switzerland

Summary

Interdisciplinary differences of lung function in elite jumping, event and endurance horses assessed by indices derived from the Single Breath Diagram for CO_2 (SBD-CO_2).

Although cardiovascular and respiratory adaptations to exercise in the equine athlete at different workload intensities are known, studies considering interdisciplinary differences in dead space ventilation and alveolar efficiency measured at rest have not been established. In the present study we investigated differences of lung function indices derived from the single breath diagram for CO_2 (SBD-CO_2) and the influence of age on these indices in show jumpers, event and endurance horses. The state of pulmonary health was evaluated by clinical examination as the results were scored and used as one of the explaining variables for statistical analysis. Endurance horses were superior in all lung function indices derived from the SBD-CO_2 compared to eventers and show jumpers. As well training quality as environmental conditions might contribute to the differences of lung function in these athletes. Age related differences of lung function were significant, with the exception of the expiratory tidal volume (VT) in event horses and the dead space/tidal volume ratio according to *Bohr* (VD_{Bohr}/VT) and an index of effective CO_2 elimination (A_1/A_2) in the group of endurance horses. This study has served to determine interdisciplinary differences and the effect of age on indices derived from the SBD-CO_2; effects which should be considered in evaluating pulmonary function an health in the resting horse.

Keywords: jumping, eventing, endurance, age, SBD-CO_2, dead space, alveolar efficiency

Evaluation der Lungenfunktion in Ruhe bei Spring-, Military-, und Distanzpferden mit Hilfe von Indizes abgeleitet aus dem „Single Breath Diagram" für CO_2 (SBD-CO_2)

Untersuchungen über die Lungenfunktion in Ruhe bei Spring-, Military- und Distanzpferden mit Hilfe von Indizes abgeleitet aus dem "Single Breath Diagram for CO_2 (SBD-CO_2)".

Über kardiovaskuläre und respiratorische Anpassungsvorgänge an Belastungen bei Pferden bestehen zahlreiche Studien, jedoch wurden bisher keine Untersuchungen über interdisziplinäre Unterschiede der Lungenfunktion in Ruhe durchgeführt. Die vorliegende Studie evaluiert die Totraumventilation und alveoläre Effizienz in Ruhe mit Hilfe des "Single Breath Diagram for CO_2 (SBD-CO_2)" und den Alterseinfluss auf die pulmonale Funktion bei Spring-, Military- und Distanzpferden. Der aktuelle Lungengesundheitszustand der Pferde wurde mit Hilfe einer klinischen Untersuchung ermittelt und bei der Auswertung der Lungenfunktionsdaten in dem gewählten statistischen Modell berücksichtigt. So konnte sichergestellt werden, dass die beobachteten Resultate der Lungenfunktionsindizes auf interdisziplinäre Unterschiede zurückzuführen sind. Die Lungenfunktion der Distanzpferde erwies sich gegenüber der bei den Spring- und Militarypferden gemessenen deutlich überlegen. Der Alterseffekt auf die Lungenfunktionsindizes war signifikant, mit Ausnahme auf das exspiratorische Atemzugvolumen bei den Militarypferden und auf das Totraumverhältnis zum Atemzugvolumen nach *Bohr*, sowie auf die alveoläre Effizienz bei den Distanzpferden. Die vorliegende Studie hat gezeigt, dass interdisziplinäre Unterschiede und Alterseffekte auf die Lungenfunktion in Ruhe bestehen und diese bei der Evaluation des Respirationsapparates, insbesondere bei vergleichenden Untersuchungen berücksichtigt werden müssen.

Schlüsselwörter: Spring-, Military-, Distanzpferde, SBD-CO_2, Totraumindizes, alveoläre Effizienz, Alter

Introduction

Performance capacity depends not only on training and recuperation, but also on constitution and a number of physical (health, condition, co-ordination) and psychological factors. Respiratory function is essential for peak performance in disciplines where maximal oxygen uptake (VO_2max) plays a crucial role for work output as e.g. in steeple chasing and three day eventing (*Straub, 1990*). Cardiovascular and respiratory adaptations to exercise in the equine athlete at different workloads are well known (*Pelletier et al., 1987; Bayly et al., 1989; Art and Lekeux, 1993; Hopkins et al., 1998*). To our knowledge, studies considering interdisciplinary differences in dead space ventilation and alveolar efficiency measured at rest have not been established.

It has been previously argued that the single breath test for CO_2 is a useful tool for studying gas exchange in humans (*Comroe, 1962; Fletcher, 1980, Fletcher et al., 1981*). *Moens (1992)* considered that the SBD-CO_2 did provide useful information on pulmonary function during anaesthesia and *Herholz et al., (2001a, b, c)* in the conscious horse. CO_2 elimination is dependent upon both lung perfusion and ventilation. If tissue CO_2 production is unchanged, CO_2 elimination can be used to monitor ventilation (V_A) and perfusion (Q). The volumetric capnogram is a plot of the expired carbon dioxide concentration or fraction thereof that is recorded as a function of the expired volume during a single breath. Graphical presentation of the plot is called the "single breath diagram for CO_2" (SBD-CO_2). A number of variables can be obtained from the SBD-CO_2 in a non-invasive way (exp. anatomical dead space fraction, dead space fraction according to Bohr's equation). Calculation of invasive variables, such as the physiological and alveolar dead space fractions, require arterial blood samples. The dead space fraction according to Bohr's equation reflects the global ventilatory efficiency, which includes the fraction of airway dead space *(Fletcher, 1980)*. The physiological dead space is believed to be a reliable index of effective dead space ventilation in men and includes the alveolar dead space fraction *(Fletcher et al., 1981)*.

The alveolar dead space/alveolar tidal volume ratio represents the degree to which alveolar ventilation (V_A) and perfusion (Q) fail to match each other *(Hardman and Aitkenhead, 1999)*.

The aim of the present study was to investigate differences in resting lung function indices derived from the SBD-CO_2 and the influence of age on these indices in show jumpers, event and endurance horses.

Materials and Methods

Animals and study design

Ten show jumpers of different warmblood breeds, 6 to 13 years old (mean 9.3 ± 2.4 years) with a body weight from 530 to 647 kg (mean 585 ± 38.5) were included in the study (4 mares, 4 stallions and two geldings). The horses performed at international levels, three of them participated at the World Equestrian Games in 1996. All show jumpers were housed in indoor boxes with no or limited periods of pasture during the year and were fed with hay and grain mixtures.

The group of event horses consisted of 13 horses (12 geldings and one mare) of different warmblood breeds, 5 to 15 years old (mean 11.5 ± 3.5 years) with a body weight from 501 to 584 kg (mean 540.5 ± 30.4 kg). All horses competed in three to four star events and four of the horses participated at the World Equestrian Games in 1996. The event horses were kept in indoor boxes with daily pasture and they were fed hay and concentrates.

Eight endurance horses, seven geldings and one mare, of different warmblood breeds as well as two Arabs constituted the third group of horses. They were 10 to 19 years old (mean 13 ± 3.2 years) with a body weight from 385 to 537 kg (mean 454 ± 50.2 kg). The horses performed at international endurance competitions, two of them participated at the World Equestrian Championships. All endurance horses were housed in loose boxes with free access to pasture or paddocks and were fed hay and a grain mixture.

The horses were trained at the Institute Equestre National Avenches (IENA) and were tested for their status of lung function during this training period on three consecutive days. During the test period all horses were kept on straw in indoor boxes and were fed hay and concentrates.

Prior to lung function testing the status of their pulmonary health was evaluated by a clinical examination and supplementary diagnostic investigations. The latter included arterial blood gas analysis, tracheobronchoscopy and cytological examination of tracheobronchial aspirates. All horses were also lunged (five minutes each of walking, trotting and galloping) to detect coughing or nasal discharge during exercise. The results of the examination were summarised using a score system described elsewhere *(Wampfler, 2001)*. The total score of each horse was used as an explaining variable for statistical analysis in order to adjust the analysis for differences in health status between horses. Testing was performed each morning on three consecutive days between 9 a.m. and 11 a.m. On each day 2–4 measurement sessions were performed with an average of 14 breaths per session. In total, 3507 breaths were analysed (average per horse: 113 breaths). The average ambient room temperature was kept at 18 °C (range: 17 to 20 °C) and humidity to 56% (range : 54 to 58%) for all experiments.

Spirometry – Capnography measuring equipment

Expiratory flow-volume curves were recorded as a function of time with a computerised ultrasonic spirometer (Spiroson Scientific®, Isler Bioengineering AG, Zürich, Switzerland). The ultrasonic spirometer was calibrated each day using a 7-litre calibration syringe and flow and CO_2 sensors were heated for 10 minutes before measurements were initiated. Capnographic measurements were performed with an IR1507 Miniature Fast Response CO_2 Infrared Transducer (Servomex®, Jarvis Brook, Crowborough, East Sussex, TN6 3DU, UK; response time 90 ms; daily calibration with a reference gas mixture (5% CO_2)). The sample collection catheter (length 30 cm; diameter 1.2 mm; aspiration rate 200 ml/min; delay 240 ms) was fixed to the ultrasonic flow sensor that approximates the nostrils at a distance of 1 cm to 4 cm, respectively, depending on the size of the horses' head. The corresponding dead space of the facemask was estimated between 50 ml and 150 ml. The results of volumetric capnography were corrected for body temperature, pressure and water saturation (BTPS) and the CO_2 volume (VCO_2) was corrected for the sampling delay of the CO_2 analyser before plotting against the expiratory tidal volume (VT).

SBD-CO_2 derived indices of lung function

The data of the recorded CO_2 and Volume curves were exported for off-line plotting of SBD-CO_2 curves and the calculation of SBD-CO_2 derived lung function indices (Oberli-Engineering, Hasle-Ruegsau, Switzerland).

The dead space according to *Bohr* (VD_{Bohr}) was calculated according to the equation:

$VD_{Bohr} = VTE\text{-}VTE * (PECO_2 / PETCO_2)$ (ml)

where $PETCO_2$ is the end tidal CO_2 pressure and $PECO_2$ is the mean end tidal CO_2 pressure, calculated from :

$PECO_2 = VCO_2/VTE$ *(Bohr, 1891)*

Bohr's equation was modified by replacing $PETCO_2$ by $PaCO_2$, the partial pressure of carbon dioxide in arterial blood. Physiological dead space (VD_{phys}) was calculated according to the equation:

VD_{phys} = VTE – VTE * ($PECO_2$ / $PaCO_2$) (ml) *(Enghoff, 1938)*. Blood is now regarded as a physiological integrator of the CO_2 pressures existing in all parts of the lung.

The dead space indices VD_{Bohr} and VD_{phys} were given as ratios' to VT.

The alveolar dead space (VD_{alv}) was calculated by

$VD_{alv} = VD_{phys} - V_{ds}$, V_{ds} being the anatomical dead space volume calculated by the pre interface expirate (PIE) method (Wolff et al., 1989). We also used the ratio of VD_{alv} to the alveolar part of the expiratory tidal volume (VT_{alv}), calculated from: $VT_{alv} = VT - V_{ds}$.

The estimation of alveolar efficiency (Alv_{eff}) is illustrated in fig. 1 and for the calculation of the ratio A_1/A_2, which is another index of efficient CO_2 elimination from the alveoli, we refer to *Herholz et al. (2001b)*.

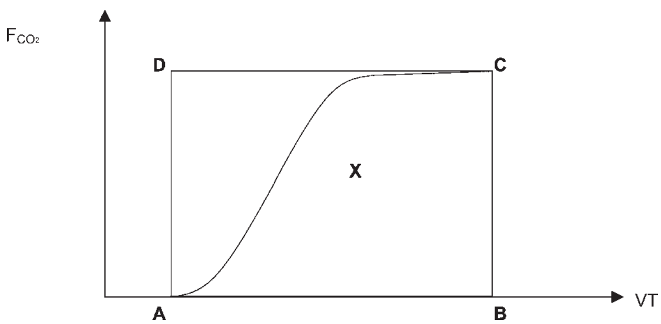

Fig. 1: Estimation of the index alveolar efficiency from the SBD-CO_2 Area X, the volume of CO_2, contained in the breath is related to area ABCDA, i.e. the volume of CO_2 that would have been eliminated by a theoretical ideal lung with the same effective tidal volume and end-tidal fraction of CO_2 (FCO_2). The term is related to *Bohr*'s original dead space concept *(Fletcher, 1980)*.

Bestimmung der alveolären Effizienz aus dem SBD-CO_2 Fläche X, das Volumen von CO_2 einer Exspiration steht im Verhältnis zur Fläche ABCDA, welche das Volumen CO_2 darstellt, das von einer theoretischen idealen Lunge mit sich entsprechendem effektiven Exspirationsvolumen und endexspiratorischer CO_2 Fraktion (FCO_2) eliminiert worden wäre. Der Ausdruck alveoläre Effizienz nimmt Bezug auf das Totraumkonzept nach Bohr (Fletcher, 1981).

Statistical Analysis

The statistical models used in this study allow unequal group sizes through adjusting the degrees of freedom. Multiple measurements can increase the precision of estimates when accounting for the variance between measurements *(Herholz et al., 2001a)*, therefore the corresponding variables (day, session, breath number within session) were included in the models as nuisance factors. The reason of analysing the effects of age nested within discipline is based on the assumption that age effects may not be at equal levels within each discipline under study. Excluding age as an explaining variable in the statistical models would result in considerably biased results because age distribution was not equal between groups.

Descriptive procedures included calculation of averages ± standard deviations. Differences of lung function indices between disciplines were analysed using a linear model which was applied for each target variable (Y).

Y = VT, VD_{Bohr}/VT, VD_{phys}/VT, VD_{alv}/VT_{alv}, Alv_{eff} or A_1/A_2

The explaining variables were:

Discipline: show jumper, event or endurance horse

Age: categorised in 2 levels (< 12 years, ⊕ 12 years)

Weight*Discipline: body weight within discipline (as nested effect)

Score: health score from clinical examination

Barometric pressure: barometric pressure at the day of the measurement

Day: day of measurement (1, 2, 3)

Session: number of measurement sessions within one day (1, 2, 3, 4)

Age*Discipline: age times discipline interaction

Differences between disciplines were interpreted as differences between least square means adjusted for all other covariables in the models. All models were estimated using the GLM procedure of SAS 8.0 (SAS Institute Inc., Cary, NC, US). Pairwise comparisons between means were performed by using the *Bonferroni* procedure. The fit between models and data was evaluated by the analysis of residuals and the assessment of the amount of variance accounted for by the models (R-Square values). The level of significance of statistical tests was set to 0.05 throughout the study.

Results

Fit between statistical models and data was satisfactory, residuals appeared to be normally distributed.

The effects of discipline, age, body weight, clinical score, barometric pressure, the testing day, the session number of measurements and age times discipline interaction were significant ($p<0.05$) for all indices studied.

The least square means of the lung function indices in show jumpers, event and endurance horses were significantly different and are listed in Table 1 and illustrated in figures 2 and 3. Effects of age times discipline interaction are shown in Table 2. The expiratory tidal volume was lower in the older horse group except in event horses. The indices of an effective CO_2 elimination decreased and the dead space indices increased with age.

Tab. 1: Least square means of lung function indices derived from the SBD-CO_2 in jumping, event and endurance horses, differences between the three disciplines were significant ($p<0.05$) for all indices

Least Square Means von Lungenfunktionsindizes abgeleitet aus dem SBD-CO_2 bei Spring-, Military-, und Distanzpferden; die Unterschiede zwischen den drei Disziplinen waren für alle Indizes signifikant ($p<0.05$)

Index	Jumping	Event	Endurance
VT (ml)	5454.64	6553.52	7542.85
VD_{Bohr}/VT	0.62	0.48	0.40
VD_{phys}/VT	0.72	0.54	0.41
VD_{alv}/VT_{alv}	0.45	0.34	0.14
Alv_{eff}	0.55	0.66	0.84
A1/A2	41.46	54.50	61.40

The only exception was VD_{Bohr}/VT and A_1/A_2 in the group of endurance horses.

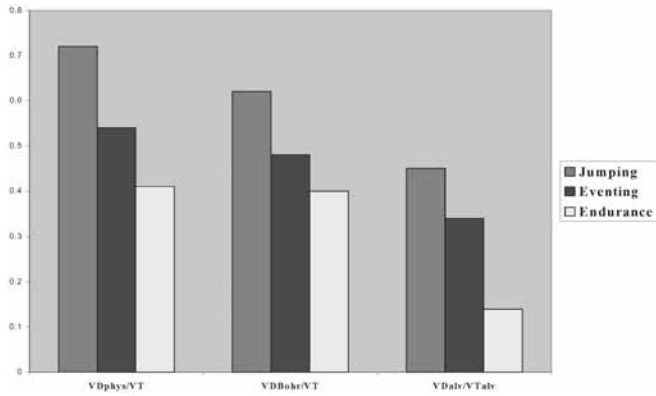

Fig. 2: Comparison of VD_{phys}/VT, VD_{Bohr}/VT and VD_{alv}/VT_{alv} (LSQM, $p<0.05$) in show jumpers (n=10), event (n=13) and endurance horses (n=8)
Abbreviations see text

Vergleich von VD_{phys}/VT, VD_{Bohr}/VT und VD_{alv}/VT_{alv} (LSQM, $p<0.05$) bei Springpferden (n=10), Militarypferden (n=13) und Distanzpferden (n=8).

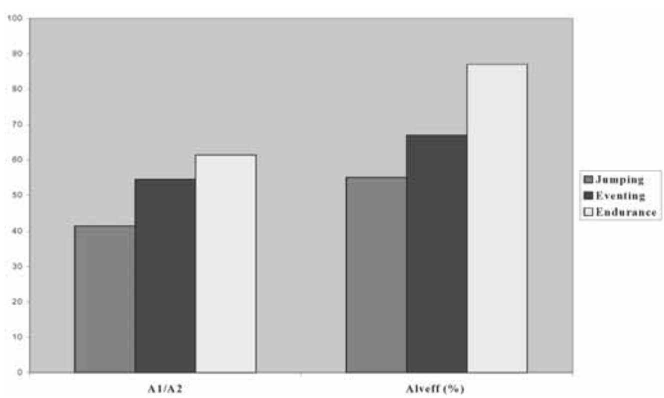

Fig. 3: Interdisciplinary differences (LSQM, $p<0.05$) of alveolar efficiency and A_1/A_2 as an index of efficient CO_2 elimination
Abbreviations see text

Interdisziplinäre Unterschiede (LSQM, $p<0.05$) der alveolären Effizienz und dem Index A_1/A_2 als Ausdruck effizienter CO_2 Elimination.

Discussion

In the present study significant interdisciplinary differences of pulmonary function in jumpers, event and endurance horses have been established by indices derived from the SBD-CO_2.
The physical jumping performance of top class show jumpers depends on strength and speed and is therefore predominantly dependent on the anaerobic form of energy production (glycolysis). Eventing consists of three disciplines: dressage (day 1), steeple chase and cross-country (day 2) and show jumping (day 3). Day 2 is the most demanding and qualifying part of the event. Stamina and the ability to cope with oxygen deficits and lactate accumulation in the tissue and blood determine performance in this phase, which is comparable with stayer disciplines in humans. The aerobic part of the work capacity and basic fitness play also an important role depending on the duration of the effort demanded. Optimal physical performance is therefore subject to the ideal blend of both the anaerobic and aerobic (cellular respiration) form of energy production. In the endurance horses which perform over long distances for much longer time and on lower work intensities the energy is derived mainly through the aerobic process *(Straub, 1990)*
Athletic training in men increases the range of CO_2 kinetics in the body, that is, the general stores of CO_2 and level of CO_2 retention. Both the threshold and sensitivity for CO_2 response appear to be connected with the effectiveness of CO_2 body excretion as well as its relative retention in the body *(Mischenko and Bulatova, 1993)*. It has been shown in men, that elite marathon athletes with extensive training show significantly (2–3 times) decreased sensitivity to hypoxia and more stable CO_2 sensitivity both in hyper- and hypocapnic range compared to stayer disciplines *(Rosenberg, 1967; Mischenko and Bulatova, 1993)*. The latter appeared to be closely connected with the neurogenic stimulation exerted by working limbs and cerebrofugal effects *(Asmussen, 1977)*. We found the endurance horses to be superior in their ability to eliminate CO_2, indicated by the indices Alv_{eff} and A_1/A_2. The index alveolar efficiency for elimination of CO_2 compares the measured expired volume of CO_2 which would have been expired by theoretical lung with the same effective tidal volume and end-tidal FCO_2. A reduction of the indices Alv_{eff} and A_1/A_2 both increase the dead space indices and vice-versa, which is consistent with the results of our study (Fig. 2, 3).
The index VD_{alv}/VT_{alv} represents the degree to which alveolar ventilation and perfusion fail to match each other. The degree of ventilation/perfusion mismatch assessed by the alveolar dead space, alveolar tidal volume ratio was also smallest in the group of endurance horses. In man endurance training induced an

Tab. 2: Effects of age times discipline interaction (Least square means) on SBD-CO_2 derived lung function indices

Effekt des Alters auf Lungenfunktionsindizes abgeleitet aus dem SBD-CO_2 innerhalb einer Disziplin

Index	Age	Jumping	Event	Endurance
VT (ml)	< 12 years >= 12 years	6156.73 4752.56	6574.20[a] 6532.84[a]	7762.31 7323.40
VD_{Bohr}/VT	< 12 years >= 12 years	0.58 0.67	0.43 0.53	0.40[b] 0.42[b]
VD_{phys}/VT	< 12 years >= 12 years	0.67 0.77	0.49 0.60	0.38 0.42
VD_{alv}/VT_{alv}	< 12 years >= 12 years	0.44 0.47	0.29 0.38	0.10 0.18
Alv_{eff}	< 12 years >= 12 years	0.56 0.54	0.71 0.62	0.90 0.82
A_1/A_2	< 12 years >= 12 years	45.22 37.70	59.15 49.80	62.34[c] 0.37[c]

[a, b, c] equal superscripts denote not significant ($p > 0.05$) differences between age categories within disciplines.

[a, b, c] nicht signifikante Unterschiede ($p>0.05$) zwischen den beiden Altersgruppen innerhalb einer Disziplin wurden mit gleichen Buchstaben gekennzeichnet

increase in the size of the pulmonary capillary bed *(Rosenberg, 1967)*. It is interesting to speculate, that the same mechanism might enlarge the pulmonary capillary bed in endurance horses, resulting in a smaller extent of ventilation/perfusion mismatch and decreased VD_{alv}/VT_{alv}.

Another aspect is, that environmental conditions haven't been included as explaining variable to correct the SBD-CO_2 derived indices, since a strong covariance between discipline and environment would have made the interpretation of these effects difficult.

Differences of lung function might therefore not only be explained by interdisciplinary differences of training, but also by the mode of stabling. All endurance horses had outdoor boxes with free access to pasture or paddocks. As all lung function indices have been corrected for the degree of pulmonary health, the results of pulmonary function tests might be results of the stabling conditions too.

We assume, that in horses observed differences between age categories in the impact on lung function indices may reflect differences in the stabling and environmental conditions during the course of life. During the first period of life up to 12 years jumping and event horses are mainly kept indoors and the majority of them travel from event to event, stay in different barns and are much more exposed to dust and respiratory tract infections. If horses are retired (age 13 years and older) they are predominantly kept on pasture and do not travel very frequently. In endurance horses the dead space index VD_{Bohr}/VT and the index A_1/A_2 of efficient CO_2 elimination differed not between the two age categories, since endurance horses perform up to age twenty and are mainly kept outdoors during the course of their whole life. The age related decline of lung function in men was reflected by tissue ageing and the composition of lung collagen *(Ricket and Forbes, 1972; Rea et al., 1982)*. Factors other than tissue aging which could account for the age related changes in humans are changes in respiratory muscle strength and weight by influencing lung emptying (Rea *et al., 1982)*. The factor weight can be excluded for the observed age related changes in our population of horses, since the results have been corrected for that variable.

In conclusion, endurance horses where superior in all lung function indices derived from the SBD-CO_2 determined at rest compared to eventers and show jumpers. The training quality and environmental conditions might contribute to the differences of lung function in these athletes. Age related differences of lung function were significant, with the exception of VT in eventers and VD_{Bohr}/VT and A_1/A_2 in endurance horses.

This study has served to determine interdisciplinary differences and the effect of age on indices derived from the SBD-CO_2; effects which should be considered in evaluating pulmonary function an health in the resting horse.

Abbreviations

A_1/A_2, index of alveolar efficiency;
Alv_{eff}, alveolar efficiency for CO_2 elimination in %; carbon dioxide;
LSQM least square means;
Q, perfusion;
SBD-CO_2, single breath diagram for carbon dioxide;
SD, standard deviation;
VCO_2, the total amount of CO_2 eliminated in a single expiration (ml);
VD_{Bohr}/VT, ratio of the dead space according to Bohr to the tidal volume;
VD_{phys}/VT, ratio of the physiological dead space to the tidal volume;
VD_{alv}/VT_{alv}, ratio of the alveolar dead space to the alveolar part of the tidal volume;
V_A, alveolar ventilation;
VO_2max, maximal oxygen uptake;
VT, expiratory tidal volume;

Abkürzungen

A_1/A_2, Index alveolärer Effizienz;
Alv_{eff}, alveoläre Effizienz der CO_2 Elimination in % Kohlendioxid;
LSQM, least square mean;
Q, Perfusion;
SBD-CO_2, single breath diagram for carbon dioxide;
SD, Standardabweichung;
VCO_2, Gesamtvolumen CO_2, das in einer Ausatmung eliminiert wird (ml);
VD_{Bohr}/VT, Totraum nach Bohr im Verhältnis zum Exspirationsvolumen;
VD_{phys}/VT,physiologischer Totraum im Verhältnis zum Exspirationsvolumen ;
VD_{alv}/VT_{alv}, alveolärer Totraum im Verhältnis zum alveolären Teil des Exspirationsvolumens;
V_A, alveolare Ventilation;
VO_2max, maximale Sauerstoffaufnahme;
VT, Exspirationsvolumen;

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Dr. C. Herholz, Fachtierarzt für Pferde

Department of Equine Internal Medicine
Faculty of Veterinary Medicine
University of Berne
CH-3012 Berne
Switzerland

Fax : 0041 / 31-631-2620
e-mail : Herholz@knp.unibe.ch