Massage or Music Meant to Be Relaxing, Result in Lowering Salivary Cortisol Concentration in Race Horses

Summary
At the beginning of the training routine, young racehorses are exposed to stressful stimuli. The aim of the study was to evaluate the influence of a relaxing massage which the horses received in the stable, and the influence of music piped into the stable, on the long- lasting stress level of the horses. 120 Purebred Arabian horses were studied. They were examined during the first racing season, which lasted for two years. At the beginning of the study, the horses were 28–31 months old. The horses were brought to Służewiec Horse Race Track (Warsaw, Poland) from their familiar studs and were randomly assigned to music (n=48), massage (n=48), or control (n=24) groups. All horses were regularly trained and competed in official races. Once a month, saliva samples were collected from each horse to determine the cortisol concentration. Both music and massage resulted in significantly lowering salivary cortisol concentration compared to the control treatment.

We see them move to music through Dressage routines. As riders, we partner with our rhythmic friends eliciting their tempos in two and three beats, walking, trotting and galloping, defining the equine species as one of nature's musicians. The equine hearing range is higher than human hearing. Our human frequency hearing range is 20Hz to 20,000Hz. The frequency hearing range of a horse is 55Hz to 33,500Hz. In fact, horses and humans share the most closely related hearing ranges than any other mammals on the planet. A whisper in their ear from your voice or a personalised whistle from the barn to come in from the field is a significant part of the deep bond with our horses and strong connection through sound. This understanding has been an integral part of the research of Pet Acoustics Inc. with the innovation of a special speaker system that modifies sound disbursement for animal hearing and specifically designed science-based music for dogs, cats and horses by Janet Marlow, internationally recognised composer and Sound Behaviourist.

In 2015, a two-year study was initiated by Witold Kędzierski1, Iwona Janczarek, Anna Stachurska and Izabela Wilk of the Department of Biochemistry, University of Life Sciences in Lublin, Lublin, Poland Department of Horse Breeding and Use, University of Life Sciences in Lublin, Lublin, Poland. The study used the Pet Acoustics speaker and Janet Marlow's equine specific music in the following study.

Introduction

Young Purebred Arabian horses in Poland are routinely submitted to race training. At the age of 2.5 years, they are moved from familiar studs to an unfamiliar race training centre. Much of the stimuli accumulated at the beginning of the training routine is associated with transport, change of residence, physical effort and participation in races, and can elicit chronic stress in horses (Alexander and Irvine 1998). Moreover, the commercialisation of racetracks alters the living conditions towards circumstances inconsistent with the biology of horses (MacTaggart *et al.* 2010). Keeping a racehorse in a box for most of the day, results in restricted freedom of movement (Henderson 2007). An unfamiliar environment, isolation, and short feed intake can also deepen the stress level (Waters *et al.* 2002). It is known that long-lasting stress has a negative influence on the organism. The factors generating stress in trained horses, however, can only be partially eliminated. Therefore, negative factors should be controlled and their effects should be mitigated (Evans 2003). Various relaxing methods may be used, e.g. free movement in the paddocks, massage and music. Massage promotes general body relaxation and increases the sense of an animal's well-being (Scott and Swenson 2009). In horses, the heart rate measured during and immediately after a massage was reduced, and improved behavioural responses were noted (McBride *et al.* 2004). Horses are generally sensitive to music. The most visible sign of the influence of music on horses is the horse's ability to synchronise their movement to musical rhythm (Bregman *et al.* 2012). According to Carter and Greening (2012), the effect of the music on a horse's behaviour depends on the music genre. Stachurska *et al.* (2015) showed that relaxation music positively affected the emotional state in racehorses. However, little is known how these kinds of relaxation methods reduce long-lasting stress in racehorses (Scott and Swenson 2009).

The most common approach used to evaluate the stress level in horses is measuring cortisol release (Peeters *et al.* 2010, Schmidt *et al.* 2010a, b). The cortisol is a natural glucocorticoid hormone synthesised by the adrenal cortex, which in turn, is stimulated by sympathetic nervous system activity. The main function of the hormone is to increase blood glucose level during effort and stressful conditions. In horses, the blood plasma cortisol concentration reflects not only the response to exertion (Desmecht *et al.* 1996, Nagata *et al.* 1999) but also the mental stress level (Cayado *et al.* 2006, Fazio *et al.* 2008). In recent years, in cortisol determination, more attention has been devoted to saliva sampling. This form of sampling is less stressful for the horse than blood sampling (Peeters *et al.* 2010, 2011). The level of the saliva cortisol correlates well with its level in the blood (van der Kolk 2001, Peeters *et al.* 2011, Bohak *et al.* 2013). The salivary cortisol concentration was successfully used as an indicator of the stress level in horses in response to stabling (Harewood 2005), road transport (Schmidt *et al.* 2010a, b) and exercise (Kędzierski *et al.* 2013, 2014a). A significant increase in the salivary cortisol concentration was stated in naïve horses during the initial training. The increase was particularly significant in response to mounting by a rider (Schmidt *et al.* 2010a, Kęd- zierski *et al.* 2014b). Thus, measuring the cortisol concentration in saliva samples was assumed to be a useful marker of mental stress in horses.

The aim of the study was to evaluate the influence of a relaxation massage, and music piped into the stable, on the horse's long-lasting stress level gauged with the salivary cortisol concentration.

Materials and methods

This article presents data collected within a larger research project designed to determine the influence of chosen relaxation techniques on the emotional state of young racehorses. The study tested the hypothesis that relaxation techniques such as massage and music used over a long period of time, decrease the salivary cortisol concentration in racehorses.

Horses
In the study, 120 Purebred Arabian horses were examined over two years: 57 horses in the first year and 63 in the second year. All the horses participated in the study during their first racing season. Each horse was studied for six months, which covered the full race season for three-year old Purebred Arabian horses. At the beginning of the study, the horses were 28–31 months old. The horses were brought to Służewiec Horse Race Track (Warsaw, Poland) from their familiar studs about three months earlier to acclimatise to the new conditions. After a month of acclimatisation, the horses began the initial training. On the first few days, they were bridled and saddled inside their boxes. Next, they were walked and trotted in an automated horse walker for 30 minutes a day. After that, the horses were mounted. The caretaker held the reins and another caretaker assisted the rider to first lay over the back of the horse and then to move to a sitting position in the saddle. This initial training lasted nine to twelve days, depending on the horse's behaviour. Finally, the riders walked and trotted the horses in a paddock for 30 minutes a day for about six weeks.

The horses were randomly divided into three groups: control group (n=24), music group which listened to music meant to be relaxing (n=48), and massage group (n=48) which regularly received a relaxing massage for about 30 minutes, three days a week. The only criterion of dividing the horses into the groups was that the groups were to include a similar number of mares and stallions. During the whole testing period, the horses were housed in four stables under comparable social and environmental conditions. Each horse was kept in a box stall measuring 3.5 m × 4 m. Straw bedding allowed the horse to comfortably lie down. To reduce the influence of nonspecific factors on the animal's emotional reaction, all the horses were fed the same diet according to dietary guidelines and cared for by the same caretakers in a manner which was typical for racehorses. This means that all horses had equal exposure to all the caretakers. To minimise the influence of the year and stable factors, the experiment was arranged in a manner shown in Table 1. For the first year of the study, the massage was introduced in stables 1 and 2, and the music in stables 3 and 4. The schedule was revised in the second year of the study. The control group also continued in respective stables in the consecutive years.

Table 1 Organization of the groups of horses

Year of the study	Stable 1	Stable 2	Stable 3	Stable 4
1st	Massage n=14 Control n=6	Massage n=10 Control n=6	Music n=11	Music n=10
2nd	Music n=14	Music n=13	Massage n=14 Control n=6	Massage n=14 Control n=6

Before the study, all of the horses were clinically sound according to a veterinarian. All the horses showed normal behaviour according to the trainer. Not all of the horses remained in training for the whole racing season because, in some cases, the owners decided to end their horse's career before the end of the season. Thus, the number in the data for each month was lower than the number of horses included in a group, and amounted to 62.5%–89.6%.

Training and Racing
The experiment was performed according to the regular race-training schedule, and for two race seasons. The training sessions were performed for about one hour a day, six days a week. The riders saddled the horses then walked the horses for approximately 10 minutes as a warm-up exercise. The primary race training was conducted on the sand track. The speed and duration were individually adapted to the level of each horse's performance. An exception was made for the measurement days. On the measurement days, each horse had to cover a distance of 1800m at a speed of 6.4m/s. After the exercise, the horses were put on an automatic horse walker for 45 minutes. At the end of the third month of the study, the horses started to compete in official races at least once a month. A day before and two days after the race, the horses were only given 60 minutes of exercise in the automatic walker. On those days, they were not examined. All persons included in the training and maintaining of the studied horses didn't know the purpose of the study.

At the end of both race seasons, the horses' performance was estimated with four parameters based on official race records: (1) number of races, in which a horse won; (2) prize per race, i.e. sum of prizes won to the number of races a horse took part; (3) success coefficient, i.e. sum of prizes won by a horse to sum of prizes won by all horses at the same age in respective race season; (4) general handicap, i.e. theoretical weight (in kg) a horse should carry in a race to equal the horse's chance to win the race, with the chances of other horses at the same age.

Listening to Music
For approximately five hours a day, the music group listened to music piped into their stalls. The music was played in the stable from 1 to 6 o'clock p.m. The music used was specifically composed and recorded by Janet Marlow, a specialist in the scientific use of music for animals. The rhythms and sound frequency of each composition were specifically composed to be within the sensitivity of the equine hearing range (Saslow 2002, Wells 2009). The relaxation music contained 10 tracks of equine-specific music, composed by Janet Marlow, Sound Behaviourist and Founder of Pet Acoustics. The music was heard in the stable through a special sound speaker device: My Pet Speaker®, Pet Acoustic Inc, Connecticut, USA) also designed to be within the range of horse-hearing. The speaker system design limits sound frequencies into 200 Hz ~

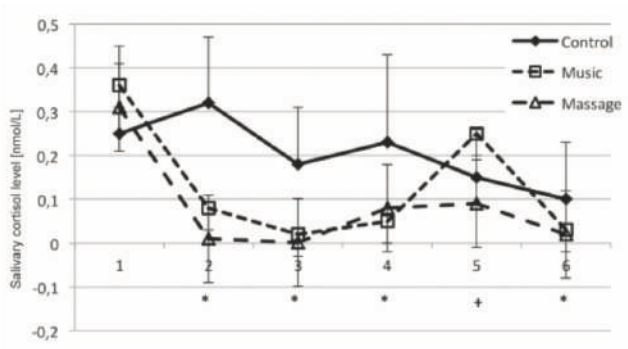

Fig. 1 Salivary cortisol level determined at rest in studied groups of horses during following months of the training season (means ± SD). 1, 2, 3 ... = following months of the study; * = means in Control Group differ significantly in comparison to other groups; += mean in massage group is significantly different than in music group.

12 KHz and features a soft bass designed for equine listening comfort. The speaker also disbursed the music in 360 degrees resonating from the four open areas of the top of the speaker. The equine music by Janet Marlow was played every day in the "Repeat All" mode. The volume was set in the mid-range; at about 65–70 decibels. The equine hearing comfort levels are between 60–80 decibels in general.

The results of the massage group were significantly lower than the results for the music group. To simplify the graphs, in both Figures, the values of SD are presented as only upper or lower bars, nevertheless, each value of SD above the mean was equal with those below the mean.

The race performance parameters are presented in Table 2. Horses in the massage group achieved better results than those in the control group with regard to the number of races won, prize per race and success coefficient. Music group had significantly higher prize per race than the control group. All the studied performance parameters did not significantly differ between music and massage groups.

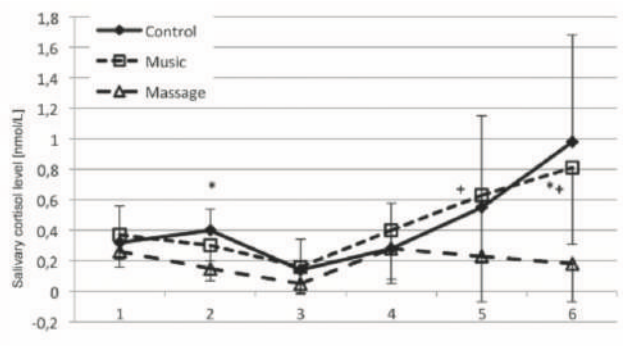

Fig. 2 Salivary cortisol level determined after the end of training sessions in studied groups of horses during following months of the training season (means ± SD). 1, 2, 3 ... = following months of the study; * = means in Massage Group are significantly different than in Control Group; + = means in Massage Group differ significantly, in comparison to Music Group.

Discussion

The results of the present study clearly show that both stress-coping methods (music meant to be relaxing in the stable and relaxing massage), brought positive effects. Introducing those methods significantly decreased the release of cortisol. The amount of cortisol was estimated on the basis of the salivary cortisol concentration. The use of this kind of stress-level estimation is commonly accepted in current research (Schmidt *et al.* 2010 a and b, Peeters *et al.* 2010).

The most interesting results concerned the determination of the cortisol level in saliva samples, taken at rest. The lack of differences in the first measurement taken at rest, showed that, as was expected, the three groups showed similar cortisol levels at the beginning of the study. The similarity of the groups was in accordance with the trainer's opinion on the normal behaviour of all of the horses. Both of the relaxation methods significantly decreased the cortisol release as compared to the control group, during the whole racing season, excluding the fifth month of the study. It is worth noticing that the massage was performed after the training sessions, so the horses were massaged at noon, and they listened to music in the afternoon. The horses were investigated at rest, in the early morning, hence the influence of the relaxation methods was analysed many hours after the relaxation treatments. Our results indicate the long-lasting effect of the relaxation methods on the salivary cortisol level in the racehorses. The positive effect the massage had on the salivary cortisol level determined just after a treatment, was described earlier (Scott and Swenson 2009). In another study, massage reduced the stress level which had been evaluated on the basis of heart rate and behaviour of the horses also during a treatment (McBride *et al.* 2004). In the fifth month of the study, the horses which listened to music had a significantly higher salivary cortisol concentration than those horses which received a massage. An analysis of heart rate variability in horses which listened to relaxation music, also showed that the effectiveness of this method decreased after some months of the treatment (Stachurska *et al.* 2015). Perhaps animals get accustomed to the music as time passes. Moreover, the horses in general get accustomed to the training. A tendency to decrease in resting cortisol values was seen in the control group during the study. Thus, probably, the salivary cortisol concentration dropped in the music group in the sixth measurement in comparison to the fifth measurement, because of a general tendency to adapt to the environment. However, the adaptation effects of long-lasting music treatment in humans and animals have been studied less. In fact, many reports describe beneficial effects of short-time use of relaxing music (Kıyıcı *et al.* 2013, Bowman *et al.* 2015, Linnemann *et al.* 2015, 2016).

The analysis of those salivary cortisol samples taken after the daily training sessions, and taken in the fifth and sixth month of the study, showed the clear positive effect of the massage treatment over the music treatment. Exercise performed by racehorses during training increases the salivary cortisol concentration (Kędzierski *et al.* 2013, 2014a), however, endurance exercise has a stronger effect (Desmecht *et al.* 1996). Some studies suggest that salivary cortisol level determined after exercise, can indicate the relative intensity of exercise in racehorses (Kędzierski *et al.* 2013). Generally, the intensity of exercise increases with the duration of training. Therefore, the values of salivary cortisol obtained after the end of training sessions tended to increase, especially in control and music groups. In the following months of the study, we compared horses which were subjected to similar amounts of intensive exercise. Thus, the differences in the salivary cortisol concentration found between massage group and other groups were the evident effect of the massage treatment. Listening to music only appears to influence mental relaxation in the horses, whereas massage has an effect on both the mental and muscle relaxation (Haussler 2009). It is not surprising that the estimated cortisol release after physical activity showed that

Table 2 Race performance parameters of the studied horses (means ± SD)

Group of horses	Number of races in which the horse won	Sum of prices to number of starts ratio	Success coefficient	General handicap
Control	0.54 ± 0.38 a	301 ± 142 a	0.90 ± 0.09 a	55.9 ± 10.5 a
Music	0.77 ± 0.29 ab	554 ± 141 b	1.36 ± 1.28 ab	61.1 ± 12.2 a
Massage	0.89 ± 0.14 b	723 ± 95.9 b	1.26 ± 0.31 b	63.8 ± 11.5 a

Sum of prices is given in Euro. Success coefficient - the sum of prizes won by a horse, divided by the mean sum of prizes won by all horses at the same age in current race season. Means in columns marked with the same letters do not differ significantly at $p<0.05$

massage brought more beneficial effects than the music. From the economic and management point of view, though, providing music is much easier than providing a relaxing massage.

Conclusion

In conclusion, both the relaxation massage and the music treatment significantly decreased the cortisol release in Purebred Arabian horses trained for racing. The massage treatment gave better results than listening to music which was meant to be relaxing. Playing music, though, being easier to provide, may be widely introduced to improve the welfare and performance of racehorses.

Funding statement

The Polish National Centre for Research and Development (grant number 180061) sponsored this study.

Citation

Kędzierski W., Janczarek I., Stachurska A., Wilk I. (2017) Massage or music meant to be relaxing, result in lowering salivary cortisol concentration in race horses. Pferdeheilkunde 33, 146-151; DOI 10.21836/PEM20170206

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Janet Marlow

Janet Marlow, M.A., Sound Behaviourist is internationally known as a researcher, composer and author. In 1997, Janet Marlow innovated a science-based method of altering the frequency and decibel levels in music, "species-specific music". This series of music for animals has been clinically proven to balance behaviours in dogs, cats, horses and birds. Her peer reviewed music studies have been published in the Journal of Equine Veterinary Science and the International Animal Health Journal. Ms. Marlow has been a consultant for Boehringer Ingelheim, (Equine) Ceva Animal Health (Canine) and Purina Friskies (Feline). In 2009, she founded Pet Acoustics Inc., an award-winning global brand with multiple products that have helped thousands of animals worldwide to diminish stress in their living environment for better animal welfare. Janet Marlow was named Woman of Influence in the Pet Industry by Pet Age Magazine. She has authored several books on animal hearing, most recently, What Dogs Hear: Understanding Canine Hearing and Behaviour.

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